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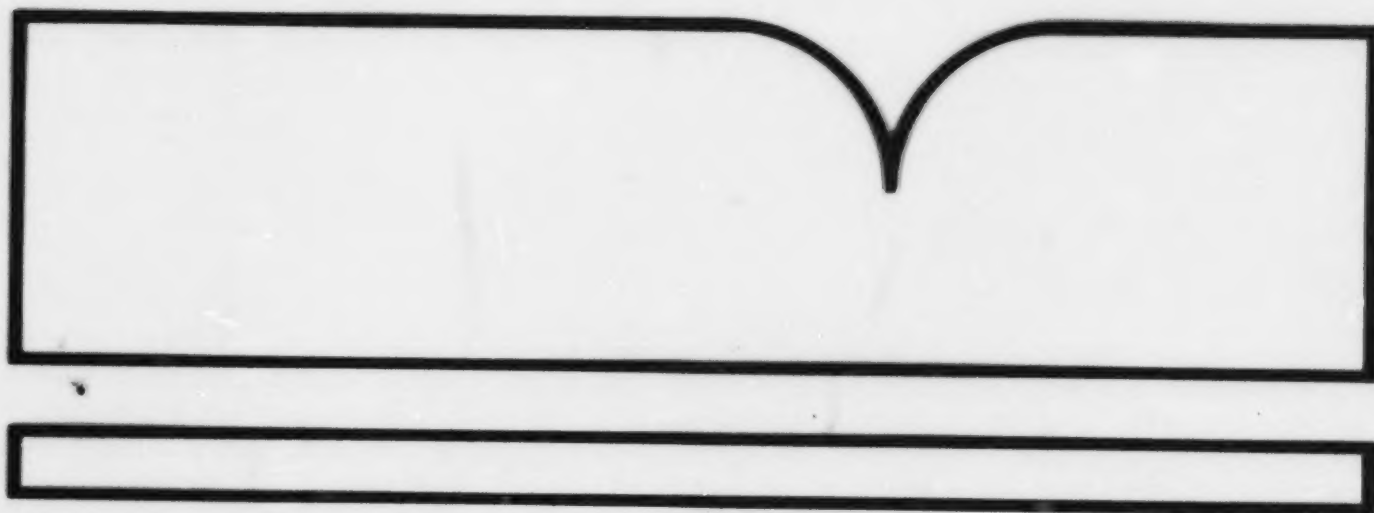
Method Development for Determination of
Polychlorinated Hydrocarbons in Municipal Sludge

Southwest Research Inst.
San Antonio, TX

Prepared for

Environmental Monitoring and Support Lab.
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METHOD DEVELOPMENT FOR DETERMINATION OF POLYCHLORINATED HYDROCARBONS
IN MUNICIPAL SLUDGE

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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Environmental Monitoring and Support Laboratory - Cincinnati conducts research to:

Develop and evaluate techniques to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.

Investigate methods for the concentration, recovery, and identification of viruses, bacteria, and other microbiological organisms in water and to determine the responses of aquatic organisms to water quality.

Develop and operate an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.

The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user-community.

The report describes the development of a method for measuring the amounts of pesticides and PCBs in municipal sludge. The method uses GC quantitation with an electron capture detector and confirms the identity of the organics with GC/MS techniques

Robert L. Booth

Robert L. Booth, Acting Director
Environmental Monitoring & Support Laboratory

Francis T. Mayo, Director
Municipal Environmental Research Laboratory

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ABSTRACT

This report describes the work performed and the conclusions obtained from a study on the development of a method for analysis of municipal sewage sludge for chlorinated pesticides and biphenyls. The methodology developed consists of extraction of the polychlorinated compounds by liquid-liquid partitioning, cleanup by removal of some interferences on a liquid chromatographic column and by precipitation of sulfur with mercury, concentration by evaporation of the extracting solvent, detection and quantification by electron capture gas chromatography, and confirmation of identity by gas chromatography/mass spectrometry.

The methodology developed was determined to provide a sound basis for the determination of polychlorinated biphenyls and organochlorine pesticides in municipal sewage treatment facilities. The detection limit attained by application of the methodology to a number of different sludges was 0.3 μg of each single component pesticide per gram of dry sludge and 3 to 15 μg per gram dry sludge for the multicomponent polychlorinated formulations studied, chlordane, toxaphene, and Aroclor 1260.

This work was performed as a portion of EPA Contract 68-03-2606.

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SECTION 1

INTRODUCTION

Since 1969, the number of pesticide formulations and their chemical constituents has continued to increase. From 30,000 formulations, made up of approximately 1,000 substances, the number has increased by more than 10,000 to over 40,000 formulations of over 1,800 substances listed by the Environmental Protection Agency (EPA). As a result of the persistence of certain of the chlorinated pesticides and the indiscriminate toxicity displayed toward some nontarget species once the pesticides have been introduced into the environment, the use of these substances has been prohibited. The ban took effect in 1972 with DDT and has since then included compounds such as aldrin, dieldrin, chlordane, and heptachlor.

Since these chlorinated compounds display a marked resistance to degradation and have been produced in such huge amounts, about 1.6 billion pounds in 1975 in the U. S. alone, many may still be found in significant quantities in the environment despite having been banned since 1972. Notable among these compounds is DDT, probably the most widespread pesticide ever used. With the exception of a herbicide, 2,4-D, no other compound has approached the amounts of DDT used over the years.

As a part of its mission, the EPA has the tasks of monitoring the levels at which these substances exist and enter the environment, controlling their levels to minimize toxic effects on nontarget species, and regulating their use so they may continue to be beneficial to man. In 1975, legal action against the EPA led to the specification of 65 classes of toxic pollutants, the so-called consent decree "priority pollutants" which include the chlorinated hydrocarbon pesticides.

The EPA subsequently expanded the list of 65 classes to 129 specific pollutants. The priority pollutants also include the polychlorinated biphenyls (PCB's), a class of compounds used in electrical capacitors and transformers, plastics, paints, and insecticides. The PCB's are very similar to the chlorinated hydrocarbon pesticides. The similarity of these compounds' properties includes toxicity toward various organisms, persistence in the environment, and response to analytical methods.

Several of the chlorinated hydrocarbon pesticides listed with the 129 priority pollutants have already been restricted by the EPA; they are: aldrin, chlordane, DDD, DDT, dieldrin, endrin, heptachlor, and lindane (γ -BHC). Others included on the priority pollutants list are: the other three BHC isomers, heptachlor epoxide, DDE, the two endosulfan isomers,

endrin aldehyde and toxaphene. The PCB's include seven major formulations: Aroclor 1016, 1221, 1232, 1242, 1248, 1254 and 1260. The last two digits indicate the percentage of chlorine present in the formulation, which is a mixture of the polychlorinated isomers of biphenyl. Aroclors, however, are no longer manufactured due to their banishment from the market effective July 1, 1979.

In order to meet the objectives of its mission, the EPA must have suitable analytical methods capable of determining the priority pollutants in air, water, and soil. The latter two media include many different mixtures or forms, each of which can cause its own special analytical problems. To meet this need, the EPA sponsors programs aimed at the development, evaluation and testing of analytical methods for application in various aspects of environmental quality programs.

There are many ways in which the organic priority pollutants can enter the nation's waterways. The chlorinated hydrocarbon pesticides may be introduced through direct application to the land, from runoff from both domestic and urban areas, and by discharge from industrial operations. The PCB's enter the aquatic environment principally through the disposal of municipal and industrial wastes in dumps and landfills, although a significant amount may have been introduced as a contaminant in industrial wastewaters.

One of the main reasons for treating municipal wastewater has been to decrease the discharge of oxygen-demanding materials into the aquatic environment. This has recently been expanded to include the reduction of potentially toxic substances not only in the finished water discharged from these plants, but also from the solid byproducts of municipal sewage treatment processes which are often used for landfill operations and, with slowly increasing frequency, as soil conditioners. The chlorinated hydrocarbons are both oxygen-demanding materials and known toxicants.

Since municipal wastewater contains not only domestic sewage but also storm water drainage, commercial wastes, and in some areas, significant amounts of industrial wastes, the chlorinated substances can find their way into municipal treatment systems in large quantities. These substances do not usually have a marked deleterious effect on the treatment processes, but they can be returned to irrigation and drinking waters where they are toxic to various organisms, including the human populace. It then behooves the authorities, and in particular the EPA, to be able to monitor the presence of these materials and to determine their fate and effects.

To remove pollutants, a majority of municipal sewage treatment facilities utilize the primary sedimentation process followed by biological activity to remove organics by contact with oxygen activated microorganisms prior to disinfection and discharge. Analytical methods are required for the determination of chlorinated hydrocarbons in both the solid and liquid discharges in order to provide information as to their effects and final disposition.

This program was initiated by the EPA at Southwest Research Institute to develop and evaluate analytical methods to be applied to municipal sludges as a complement to ongoing programs designed to provide analytical methods for water and wastewater. Specifically, this program was to provide a method for the qualitative and quantitative determination of the priority pollutant polychlorinated biphenyls and organochlorine pesticides in municipal sewage treatment plant sludges.

The analytical method was to include a means for isolating the chlorinated substances from sludge and for detecting the individual compounds at a minimum concentration level of 300 ppb in the dry solids residue. The method also was to provide a means for removing analytical interferences and for confirming the identity of any of the subject compounds detected.

The method developed as a result of this study includes extraction of the polychlorinated compounds by liquid-liquid partitioning, cleanup by removal of some interferences on a liquid chromatographic column and by precipitation of sulfur with mercury, concentration by evaporation of the extracting solvent, detection and quantification by electron capture gas chromatography, and confirmation of identity by gas chromatography/mass spectrometry.

SECTION 2

CONCLUSIONS

The results of experimental studies reported here have led to the formulation of an interim screening method for application to municipal sewage treatment plant sludges for compliance with the NPDES Permits Program. The method includes the following major steps:

1. extraction of the polychlorinated compounds from sludge by homogenization with a mixture of organic solvents followed by centrifugation;
2. isolation of the chlorinated compounds from some interferences by;
 - a. gel permeation chromatography to remove interfering organics, and
 - b. removal of elemental sulfur by shaking with metallic mercury;
3. concentration of the chlorinated compounds to within the separation and detectability range of the determination method;
4. quantitative determination of polychlorinated compounds by gas chromatography/electron capture detection; and
5. confirmation of the identity of detected compounds by gas chromatography/mass spectrometry.

The minimum detectability limit attained is 0.3 μg of each single component pesticide per gram of dry sludge and 3-15 μg per gram dry sludge for the multicomponent polychlorinated formulations. These limits are based on observations of a small number of different sludges and may be subject to change as information is acquired from varying types of sludges.

The data collected on this program provide a basis for further experimentation to statistically characterize the reliability of the method, improve the various procedures, and to make further developments for qualification as a verification method. Refinement of the method will be required prior to its application to studies more rigorous than screening surveys.

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SECTION 3

RECOMMENDATIONS

The various elements of this procedure should continue to be investigated. Whether this method meets the requirements of general applicability, statistical reliability, and economic considerations must be evaluated by the appropriate governmental agencies. The method provides a sound basis for the determination of the fate of the polychlorinated biphenyls and organochlorine pesticides in municipal sewage treatment facilities.

Further development of the procedure is required in order to produce a method that provides more reliable qualitative and quantitative analyses. Specifically, these studies should be continued and expanded to:

1. enhance the accuracy and precision of extraction techniques;
2. explore the modification of existing methods and/or use of other methods to enhance sample cleanup and remove more substances which interfere with determinative procedures;
3. develop the application of electronic integration and computer data handling methods to provide faster, more economical, and more accurate calculations;
4. investigate other gas chromatographic detectors which may enhance selectivity and obviate the need for more cleanup in the quantitative procedures;
5. compile mass spectral response data of reference compounds in sludge and form a data bank to make the method more applicable on a mass scale;
6. characterize sludges from facilities in different geographic areas which have varying influent loads from runoff, industrial, and domestic sources;
7. integrate the method into an overall procedure for the determination of 129 consent decree organic priority pollutants.

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SECTION 4

EXPERIMENTAL PROCEDURES

GENERAL PROCEDURES

This program was initiated on a quick reaction basis in June 1978 to provide an interim method by December 1978 which could be used by the EPA for the NPDES Permits Program as it applies to municipal sewage treatment plants. The program was continued in order to further evaluate and refine the interim method proposed in December 1978. The experimental effort covered in this report extended through the period June 1, 1978 to December 31, 1979. Techniques and procedures were chosen for further study from those already available for water and wastewater after an assessment as to their stage of development and the probability that they could be successfully applied to municipal sludges. Consequently, most of the techniques and procedures were tested on a concurrent basis requiring a significant amount of duplication of effort. Electron capture gas chromatography was chosen as the quantitative step to be used throughout the program since it exhibits a high degree of selectivity and detection sensitivity for chlorinated hydrocarbons, has been the method of choice for many similar studies involving diverse sample matrices, and is an economically attractive approach. Gas chromatography/mass spectrometry was chosen for qualitative confirmation of the chlorinated organic compounds.

The various elements of the experimental program conducted for this study were choice of sludge, determination of the solid residues, composition of the standard solutions, comparison of extraction and cleanup procedures, and application of analytical methods for determination and identity confirmation of the polychlorinated compounds.

CHOICE OF SLUDGE

For several years, Southwest Research Institute personnel have collaborated with City of San Antonio officials on programs of several types, many of them of an environmental nature, and a good reciprocal working relationship has resulted. It was a simple matter, then, to make arrangements to obtain sludge from the city's Rilling Road sewage treatment plant.

Two types of sludge, primary and digested, were sampled by SwRI personnel in 3-4 L batches and stored at 4°C in the sampling containers. The containers were wide mouth glass jars which had been cleaned prior to

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sampling by detergent washing, solvent rinsing, and drying at 400°C. The caps were screwed on over a 0.02-mm Teflon film to prevent contamination from cap liner materials.

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Later in the program, when it was desired to test the methodology with sludge from an industrial area, digested and combined sludge samples from Dayton and primary sludge samples from Cincinnati, Ohio were obtained. Three to four-liter samples were shipped to these laboratories on several occasions. An excess of primary sludge from Muncie, Indiana on hand from another program was used occasionally. The sludges were shipped under refrigeration to San Antonio where they were stored under refrigeration in the dark.

RESIDUE DETERMINATION

The method for determination of total residue was adapted from "Standard Methods for the Examination of Water and Wastewater," 14th edition. It was necessary to continuously agitate a large amount of sludge while sampling in order to assure that a representative sample was taken. A homogeneous sample of wet sludge was weighed into a clean, tared evaporating dish and placed on a steam bath to evaporate the water. The evaporated sample was then transferred to a drying oven for final drying at 103-105°C. The residue weight was determined on the cooled and desiccated sample.

SAMPLE AGITATION

In order to ensure homogeneity of samples throughout this study, several ways of mixing the solids and liquids in sludge were investigated. Several devices were on hand and were used including a high-speed propeller-type paint mixer, a paint shaker, a Gifford-Wood industrial high-speed, high-shear homogenizer, and a hand-held stirring paddle. None of these methods, by itself, was very effective. Only the industrial homogenizer broke up all of the large pieces and resulted in a fairly uniform particle distribution. The problem with the Gifford-Wood instrument was that it whipped air into the sludge, creating a froth which made it difficult to remove a representative sample as the entrained air escaped.

The agitation method used to mix the sludge samples consisted of a combination of techniques which seemed to provide the best opportunity for producing truly homogeneous samples. The sludge was homogenized with the Gifford-Wood unit, allowed to stand undisturbed for the entrained air to escape, and then agitated with a propeller-type stirrer while sampling. This was an effective procedure as long as the stirring was done carefully so the solids were kept evenly distributed but no air was whipped into the suspension.

GAS CHROMATOGRAPHIC DETERMINATIONS

The quantitative gas chromatographic determinations made in this study were all done using the same system. The gas chromatograph was equipped

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with a 1.8 m long x 4 mm ID glass column packed with 1.5% SP-2250/1.95% SP-2401 on 100/120 mesh Supelcoport.® The helium carrier gas flowed at a rate of 60 mL/min through the column which was maintained at 200°C. Injection was on column at 225°C and detection was by linearized nickel-63 electron capture at 300°C. Electron capture is highly sensitive to polychlorinated compounds such as those included on the priority pollutants list and studied here.

The individual compounds may be detected in amounts of a few tens of picograms (10^{-12} g). The required minimum detectability of 300 ng in 1 g of solids could then be achieved by extracting 20 g of a 5 percent solids sludge, concentrating the extract to 10 mL and injecting 10 μ L which would contain 300 pg of any compound present at the 300 ppb level in the solids. This would be adequate for any single compound, but it would be quite difficult to detect the multicomponent formulations, chlordane, toxaphene, and the Aroclors.

Information from the wastewater phase of this contract dealing with the polychlorinated compounds indicated that minimum detectability expected for chlordane would be about 3 μ g in 1 g of solids under the conditions stated above. Minimum detectability expected for toxaphene would be about 15 μ g in 1 g solids and for AR 1260, the PCB chosen for these studies, about 6 μ g in 1 g.

Authentic reference standards of the 17 formulations were obtained from the EPA Health Effects Research Laboratory at Research Triangle Park, North Carolina. These compounds were dissolved singly and in mixtures at appropriate concentrations and their gas chromatographic retention times were determined. The 17 chlorinated organic compound formulations were separated into five groups for convenience of handling. Group I was made up of α -, β -, and δ -BHC, p,p'-DDE, p,p'-DDD, p,p'-DDT and heptachlor epoxide. Group II included the compounds γ -BHC, aldrin, endosulfan I and II, dieldrin, endrin and its aldehyde, and heptachlor. The multicomponent formulation, chlordane, makes up Group III, and Group IV is made up of the many compounds which together constitute the pesticide, toxaphene. The multicomponent pesticides are quite complex and not always easy to recognize. Batch to batch variations of the relative amounts of the compounds in these formulations can also serve to complicate their analytical recognition and quantitative determination as can any compounds that elute within the retention time span of the compound in either chlordane or toxaphene. An analyst attempting to determine these compounds in a complex matrix such as municipal sludge must be thoroughly familiar with the response of toxaphene and chlordane under a variety of conditions and be able to recognize them when they are apparently not present. Access to gas chromatographic/mass spectrometric instrumentation is preferred as it makes the identification of these mixtures more reliable.

The final mixture is Group V, Aroclor 1260, a formulation of a number of polychlorinated biphenyls which together have a chlorine content of 60 percent indicating a high degree of chlorine substitution. The PCB's are

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made up of compounds which are very similar to those of the pesticides. For this reason the PCB's and pesticides are mutual interferences and it requires a highly experienced analyst to be able to recognize one in the presence of the other. The PCB's and the pesticides are subject to interference from other electron capture responsive substances found in the sample matrix. Although Aroclor 1254 was one of the most used PCB's, we chose to work with Aroclor 1260 for this study because it includes most of the compounds contained in Aroclor 1254 and enough of the more highly chlorinated compounds to extend the gas chromatographic region beyond that of the pesticides. Furthermore, the properties of the two formulations are very similar, making them interchangeable for analytical purposes.

PCBs

Flame ionization (FID) and Hall Electrolytic conductivity (HECD) detectors were also used for this study. The FID was used with a 30 meter SP-2250 SCOT column in an attempt to further characterize the sludge extracts and obtain more information on their organic content. The HECD was used with the mixed phase pesticide column described earlier in this section. This detector was operated in the halogen specific mode in order to provide additional information which could be used for confirmatory identification of the organochlorine compounds.

A second liquid phase column material, OV-330, was also investigated as a possible means of providing additional qualitative information useful to the identification of the organochlorines. The OV-330 was used both alone and in combination with SP-2250 in glass columns identical to those used for the quantitative work. Detection was by electron capture for the three liquid phase loadings 4% OV-330, 2% OV-330 + 3.5% SP-2250, and 1.7% OV-330 + 1.7% SP-2250 coated on 100/200 Supelcoport support phase.

EXTRACTION METHODS

The extraction procedures in an analytical method can often be the most critical in terms of overall reliability. The ideal extraction would remove only the analyte compounds and, without additional manipulations, provide them in the concentration necessary for analytical determinations. This, of course, is seldom the case. Municipal sludges, because of the complexity of compounds they contain present an extreme deviation from the ideal. The nature of these compounds in sludge provides a certain advantage to extraction by partitioning into a water immiscible organic solvent. Many of these compounds are of a fatty, oily, or waxy nature and would be expected to selectively dissolve compounds such as the polychlorinated hydrocarbons. This mixture would then be present as a distinct phase in the system, whether separated from the water or suspended in it, and should partition readily into an extracting organic solvent.

With this in mind four extraction methods were chosen for evaluation: 1) liquid-liquid partitioning between immiscible solvents; 2) percolation of an organic solvent over sludge which had been rendered granular and free flowing by the addition of a salt with a high capacity for taking up water; 3) Soxhlet extraction of a sludge granulated as in 2; and 4) continuous

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liquid-liquid extraction in a modified Soxhlet apparatus. The latter two methods require continuous heat input and raise the question of the possibility of reaction or degradation of the analytes. This was not a major concern since continuous extractions under reflux have been used successfully for the extraction of polychlorinated compounds in other systems.

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Liquid-liquid partitioning was first tested by shaking a 20 g sample of sludge with dichloromethane (DCM) in a separatory funnel. An emulsion formed which was extremely difficult to break even when the ratio of solvent to sludge was increased to 25:1. This method held little promise unless some means of breaking the emulsion and separating the liquid phases could be provided. It was found that centrifugation would effectively break an emulsion formed by sludge with either DCM or hexane. Since it is more dense than water, the DCM settled to the bottom of the centrifuge tube and had to be removed by inserting a pipet, large syringe needle, or aspirator tube through the aqueous and solids layers. Usually, a small amount of the sample matrix was withdrawn with the solvent.

It was decided to use a solvent mixture of hexane, DCM, and acetone for further experimentation with the "centrifuge method" of extraction. In addition to being a solvent for the polychlorinated compounds, hexane acts as the vehicle for the extracting solvent, DCM enhances the solvation properties, and acetone promotes invasion of the interstices of the solid matrix.

The stepwise procedure for evaluation of the centrifuge method of extraction follows:

1. Weigh a 20 g sludge sample into a 200 mL Pyrex screw-cap centrifuge bottle.
2. Add 20 mL H₂O saturated with NaCl.
3. Add 60 mL hexane-DCM-acetone (83:15:2) and seal the bottle with a Teflon-lined cap.
4. Shake the bottle vigorously for at least 1 min by hand or with a mechanical homogenizer such as the Tekmar Tissuemizer® or Brinkman Polytron®.
5. For phase separation, centrifuge at 500 x G for 20 min at 15°C.
6. Carefully pipette or aspirate the upper solvent layer and transfer to a suitable receiving vessel.
7. Repeat steps 3 through 6 twice with 60 mL volumes of extracting solvent.
8. Combine the solvent extracts and retain for further procedures.

The column elution extraction method was adapted from methods used in these laboratories for the extraction of pesticides and PCB's from solids and sediments. The extracting solvent had to contain a water miscible solvent

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such as acetone in order to provide intimate contact with the water occluded within the pores of the solid matrix. A mixture of 20% acetone in hexane was chosen for evaluation of the column elution method of which stepwise details follow.

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1. Weigh a 20 g sludge sample into a beaker.
2. Add 80 g anhydrous, granular Na_2SO_4 and mix well until the mixture is free flowing.
3. Transfer the granular mixture to a 20-mm diameter glass column having a glass wool plug at the bottom.
4. Wash the beaker with the extracting solvent, 20% acetone in hexane, into the column continuing until a total of 150-mL solvent has been added.
5. Collect the eluate in a suitable vessel and retain for analytical determinations.

Extraction of granulated sludge with a Soxhlet apparatus is based on principles similar to those of the column elution method with two differences in the methodology. The extraction can be continued over long periods of time since the solvent is continuously recycled by refluxive distillation through a sample matrix, ostensibly enhancing solvation of the analytes. Also, a water-miscible solvent is not used as it would carry over a large volume of water into the extract because of the continuous cycling action of the technique. The procedural details of the Soxhlet extraction method follow:

1. Weigh a 20 g sample of sludge into a beaker.
2. Add 180 g anhydrous, granular Na_2SO_4 and stir until the mixture is free flowing.
3. Transfer the granulated sludge to a glass extraction thimble having a plug of glass wool on the frit and place another plug over the sample.
4. Charge the extractor flask with 200 mL 15% DCM in hexane which has been used to wash the beaker which contained the sludge.
5. Activate the heat controls and run for four hours at a cycle rate of 8-10 cycles/hours.
6. Retain the extract for further analytical procedures.

The continuous liquid extraction method began with a handicap that could not be overcome. The method requires the extracting vessel provide some means of mixing the sample and extracting phases on a continuous basis. This type of apparatus was not available in this laboratory and could not be obtained on a timely basis. Several Soxhlet apparatuses were modified to return the extracting solvent to the still-pot without siphoning by raising the siphon tube connection on the return tube. In this manner, extracting solvent would flow continuously through the return

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tube as it condensed and dripped through the sludge. The procedural details for this method are:

1. Place a plug of glass wool in the return tube where it connects to the extractor body. This will keep entrained solids from flowing into the distilling flask.
2. Place 175 mL DCM in the body of a modified Soxhlet extractor collecting any overflow in the distilling flask.
3. Weigh 20 g sludge into a small beaker.
4. Transfer the sludge to the extractor washing three times with 10 mL water and finally with 10 mL DCM.
5. Turn on the heat to the still-pot and extract 16 hours (overnight).
6. Retain the extract for further analytical procedures.

A commercially available material was also evaluated for drying and granulating the sludge samples for use with the column elution and continuous liquid extraction methods. The granular material is contained in the Analytichem Extube®, a hollow cylinder, and is intended to sorb an aqueous sample containing organic contaminants. The organics are then eluted by percolation of a suitable solvent such as DCM through the column. The tubes are available in basic, neutral, and acidic form.

The 20 g sludge sample was adjusted to a pH of 2-3, 6.5-7.5, or 10-11, with an inorganic buffer prior to being poured onto the appropriate Extube® material contained in a beaker. Half the sorbent material in a tube was adequate for drying 20 g sludge which was added slowly and with continuous stirring to keep the mixture from solidifying. The sample was stirred until completely dry and free flowing and then entered into the appropriate extraction procedure.

These procedures were then evaluated for extraction efficiency by spiking sludge with known quantities of authentic standards, extracting by the listed methods, removing interferences and analyzing by the procedures which follow. Also, the effect on extraction efficiency of shaking or blending the sludge/solvent mixture in the Centrifuge Method was determined, and two types of blenders, the Tekmar Tissuemizer® and the Brinkman Polytron® were compared.

CONCENTRATION AND CLEANUP

The sample extracts require concentration prior to other operations, such as cleanup or analysis. Concentration is normally achieved simply by evaporating part of the extracting solvent, usually in a Kuderna-Danish apparatus on a steam bath. Concentration to volumes less than 5-8 mL cannot be carried out effectively on a macro Kuderna-Danish apparatus. This type of concentration can be carried out by directing a stream of dry, inert gas onto the extract solution and evaporating the solvent to the

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desired volume. An alternate method of concentrating to small volumes is the use of a micro Kuderna-Danish apparatus, although caution must be exercised as this method can result in significant analyte losses. Evaporation to volumes less than 5 mL is avoided, as it often involves losses of the analyte compounds. It is required for such operations as gel permeation chromatography and great care needs to be exercised especially to prevent the solution from going to dryness.

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The chlorinated solvent, DCM, used in most of the solvation/extraction operations, interferes with use of the electron capture detector. A large amount of DCM causes a detector response which interferes with the determination of early-eluting chlorinated compounds. DCM is removed from the solution by adding 25 mL hexane to the 5-8 mL concentrate in the Kuderna-Danish apparatus and evaporating again on the steam bath.

Interferences are usually removed from the concentrated extracts prior to gas chromatographic analysis. Two methods for the removal of organic compounds and three for the removal of elemental sulfur were tested. Organic interferences were removed by liquid chromatographic fractionation on Florisil and by steric exclusion chromatography on Sephadex LH-20 or Bio-Beads S-X2 and S-X3. The removal of elemental sulfur was studied by contact with activated copper metal or elemental mercury and by conversion to thiosulfate for removal by dissolution in water/alcohol.

Florisil-PR, which is used for chromatographic cleanup procedures, is purchased activated at 675°C, is heated at 600°C for two hours prior to use. Five percent by weight water is added to the Florisil after cooling and allowed to equilibrate for 24 hours while periodically vigorously shaking the container. Twenty grams of the H₂O-deactivated Florisil is charged in a 20 mm diameter glass column and settled by tapping the column gently. A 10-15 mm layer of anhydrous, granular Na₂SO₄ is added and the column is washed with 75 mL hexane which is discarded. The sample extract is quantitatively transferred in 10 mL hexane to the column and the solution allowed to just disappear at the bottom of the sulfate layer.

The first fraction is eluted with 150 mL 6% diethyl ether in hexane and the second with 150 mL 50% ether/hexane. The fractions are collected in a Kuderna-Danish flask in preparation for evaporation of the solvent prior to other operations of the analytical protocol being conducted. All of the polychlorinated priority pollutants of interest here elute in the 6% ether fraction except for endosulfan II and endrin aldehyde which elute in the 50% ether fraction.

Both Sephadex LH-20 and Bio-Beads S-X2 and S-X3 were evaluated for removal of high molecular weight interfering materials from the sludge extracts. After original work showed the Sephadex LH-20 to exhibit some adsorption in addition to steric exclusion properties, the Bio-Beads gel was used exclusively. The procedures used for fractionation on these gel materials follow.

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To prepare either the Sephadex or Bio-Beads gel material for use, weigh 25 g into a beaker, add 2-3 volumes of cyclohexane, cover and let stand overnight. Transfer the swollen gel to a 2 mm diameter glass column equipped with a stopcock and allow to settle to a bed volume of approximately 50 cc. Flush the column with 75-100 mL cyclohexane under an inert gas pressure of 62 kPa being careful not to let any of the gel dry. Allow the cyclohexane to drain flush with the top of the gel bed and transfer 8 mL of the sludge extract to the top of the gel bed. Wash the sample vial and sides of the column three to four times with 2-4 mL of cyclohexane and allow the solvent to drain to the top of the gel bed. Fill the column with cyclohexane and elute at 5 mL/min under an inert gas pressure of 62 kPa and collect 25 mL fractions in Kuderna-Danish concentrators. Evaporate each fraction to a volume less than 5 mL, transfer in small portions to a 0.5 mL screw cap vial and maintain a stream of pure, dry inert gas on the solution until all has been transferred and it has been evaporated to 0.1 mL. Seal with a metal foil- or Teflon-lined cap and store at 4°C.

This cleanup method was used for the GC/MS confirmation of identity of compounds which had been determined by GC/ECD. Another method was developed with Bio-Beads S-X2 gel which provides a fairly sharp separation between high molecular weight compounds, which are discarded, and polychlorinated compounds which elute in a relatively small volume.

The S-X2 gel is prepared for use by weighing 30/40 g into a beaker, adding two to three volumes of DCM, and allowing to stand sealed overnight. Then transfer the swollen gel in portions to a 22 mm diameter, 400 mm long glass column equipped with a Teflon stopcock plug and allow it to settle until a stable bed height of 300-310 mm is achieved. Allow the DCM to drain flush with the top of the bed being careful not to let any part of the gel dry. Place a 15-20 mm layer of anhydrous, granular sodium sulfate at the top of the gel column and add enough DCM to cover it.

The sludge extract is concentrated by Kuderna-Danish evaporation to 5-10 mL, 75 mL DCM is added and the evaporation is repeated to a final volume of 2-3 mL. The body of the concentrator is washed with a small volume of DCM into the collection tube and final concentration is done by placing the tube in a beaker of 35°C-40°C water and directing a stream of dry, inert gas onto the solution until a final volume of 2-3 mL is achieved.

Carefully drain the excess DCM in the gel column into the Na₂SO₄ until just above the gel and allow to drain to the top of the gel bed. Rinse the sample tube with 2-3 small DCM volumes and pour into the column. The sample volume plus the rinsings should not exceed 5 mL. Allow the DCM to elute to the top of the gel layer, add 1-2 mL DCM and repeat. Add DCM to the column, begin the elution, and maintain the DCM at a level 20-40 mm above the Na₂SO₄.

Under these conditions the DCM will flow at a rate of approximately 3 mL/min. Collect the first 90 mL of eluate, which contains a large part of

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any interferences, and discard. Collect the next 50 mL containing the polychlorinated compounds, seal the container and store refrigerated. The S-X3 gel is prepared in the same manner as the S-X2. The polychlorinated compounds begin to elute earlier from the S-X3, and the discard fraction is cut off at 85 mL. The next 50 mL, as from the S-X2 gel, contains the analyte compounds.

A commercially available device, the Waters Sep-Pak® isolation and cleanup cartridge, was also evaluated for application to the cleanup of sludge extracts. The Sep-Pak® contains a sorbent material in a small tube designed such that a hypodermic syringe may be attached to one end. The syringe is used as the solvent reservoir and pump causing the solvent to flow through the column by application of pressure on the syringe plunger. These cartridges are intended to eliminate the need for large volumes of solvent during isolation and cleanup stages of analytical methods.

The silica Sep-Pak® contains silicic acid and is intended for fractionation procedures analogous to the Florisil cleanup. The column was charged with 5 mL of a concentrated extract in hexane and then followed with 5 mL 50% diethyl ether in hexane, the cleaned up chloroorganics supposedly appearing in the latter fraction.

The C18 Sep-Pak® contains a bonded hydrocarbon sorbent and is intended for a reversed phase type fractionation. The concentrated extract was placed on the column in 5 mL methanol. This was followed with 5 mL 50% diethyl ether in hexane and finally with 5 mL hexane. Unwanted compounds should be contained in the methanol fraction and the chloroorganics in the other two fractions.

CONFIRMATION OF IDENTITY

Extracts of municipal sewage treatment plant sludge normally contain a complex mixture of components and will contain a large number of compounds which will cause a response of the electron capture detector. Consequently, a positive response at a point corresponding to the elution of one of the standard compounds cannot be considered absolute proof that such a substance is present. Electron capture response in combination with retention times matching those of standards can be considered no more than an indication that any given organochlorine compound is present. The problem is not only compounded by the number of compounds that respond to the ECD in organic sludge extracts but by the presence of any of the multicomponent organochlorine formulations. Gas chromatography/mass spectrometry provides an ideal means for confirmatory identification of those polychlorinated compounds presumed to be in municipal sludge extracts from gas chromatographic/electron capture detector data.

The presence of one of these formulations will always complicate an analysis unless it is in such a large amount that it completely overwhelms any other compounds present. This problem is further complicated by the possibility of the degradation of individual compounds in the

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multicomponent formulations in such a way that a drastic change is caused in the pattern response. Pattern recognition is an important tool in the identification of a multicomponent formulation and disruption of the pattern forces the analyst to look elsewhere for identity information. The ideal place for this type of information is at the molecular level.

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The GC/MS determinations were made on the single fraction from S-X2/DCM cleanup and, alternatively when required, on the six to eight fractions of sludge extract collected from S-X2/cyclohexane chromatography. The instrument used was a U-column gas chromatograph interfaced with a molecular glass jet separator to an electron impact, quadrupole filter mass spectrometer. Data were recorded and analyzed by a computer data system. The instrument is manufactured by the Finnigan Corporation, Sunnyvale, California.

Approximately 3 μ L of concentrated extract fraction of sludge was injected for each gas chromatographic run. The chromatographic conditions were set to duplicate the results of the electron capture determinations, but if this did not provide useful data, then the extract was chromatographed by a column temperature program designed especially for it. The same column material was used in a 1.6 m long, 2 mm diameter column. The overall procedure was followed as closely as possible to that from the EPA manual, "Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants," April 1977.

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SECTION 5

EXPERIMENTAL RESULTS

GENERAL PROCEDURES

The first experiments conducted were the comparisons of extraction methods on sludges from the San Antonio Rilling Road Sewage Treatment Plant spiked with known amounts of polychlorinated standards. One extraction method was chosen and two methods of chromatographic cleanup were compared, while three methods of removing elemental sulfur were compared. All the quantitative determinations were done by electron capture gas chromatography. When all the individual parts of the analytical procedure were chosen, gas chromatography/mass spectrometry was investigated for the confirmation of identities. The integrated method was also tested for its applicability to sludges from plants in Dayton and Cincinnati, Ohio, which were expected to have a larger contribution of industrial wastes than the San Antonio sludge.

RESIDUE DETERMINATIONS

In order to be able to take representative samples of sludge from the main sample, we felt it was necessary to homogenize prior to sample division. The Gifford-Wood homogenizer made this difficult, as it whipped air into the sludge and produced a froth. Apparently, this caused the solids to stratify in suspension as a function of settling rates determined by physical characteristics such as size. As an example, a three liter sample of San Antonio primary sludge was homogenized for one hour and samples were taken from the top and bottom of the suspension. The total residue determination of samples averaged 0.8% solids at the bottom and 3.2% at the top of the container. A similar determination made on a digested sludge agitated with a paint mixer resulted in a bottom to top range of 6.6-9.4% total solids.

The procedure we settled on to provide representative sampling of sludge consisted of a homogenization with a Gifford-Wood instrument and agitation during sampling with a propeller-type stirrer after any entrained air has been allowed to escape. The determination of total solids of the San Antonio sludges in this manner resulted in the values:

Primary sludge = 3.56, 3.55, 3.66%
Digested sludge = 3.80, 3.77, 3.87%

Sludges from Dayton, Cincinnati, and Muncie were also used periodically, as they became available, to compare total solids determinations following agitation by homogenizer only and followed by stirring. The results of all the total residue determinations performed are shown in Table 1 as the average with standard deviation of three determinations each.

There are apparently no differences between the values determined by the two procedures for each type of sludge. Analysis of variance of the data confirmed this observation. There is little reason to expect any significant source of error in either of these two procedures except for any large pieces of solids in a sludge sample that would not be broken up by a propeller stirrer. Large pieces could bias results toward either higher or lower values, depending not only on whether they are included in an analysis but also on their physical characteristics such as density. For these reasons, all sludge samples were homogenized with the Gifford-Wood instrument upon arrival in the laboratory and prior to storage.

GAS CHROMATOGRAPHIC DETERMINATIONS

The five groups of analytical reference standards were gas chromatographed on the SP-2250/2401 column under identical conditions. The chromatograms in Figure 1 show that all compounds in Groups I-IV elute in less than 20 minutes. The PCB's are the exception since it can take as long as 35 minutes for all of the major compounds in these mixtures to elute.

Since detection by electron capture is not an absolute form of identification of a compound, a second chromatographic column system was sought. This second system would have to be of a significantly different polarity when compared to the one normally used and cause the transposition of some of the organochlorine compounds being studied. A liquid phase which may provide these properties is OV-330, a silicone-carbowax copolymer. The OV-330 as a single phase causes the transposition of several retention times but more than doubles the run time, especially if the PCB's are being run.

We were able to decrease run times by decreasing the amount of OV-330 used and mixing it with SP-2250. The retention times for both the SP-2250/SP-2401 column normally used and the SP-2250/OV-330 experimental column are compared in Table 2. There are major changes in the order of elution of heptachlor, aldrin, β -BHC, and δ -BHC as well as transpositions between dieldrin and DDE and endosulfan II and DDD. Since these changes of elution times are significant, the assignments for peaks in the multicomponent formulations can only be tentative. For the most part, these assignments were made arbitrarily because they corresponded to some degree in elution time and magnitude of response obtained on the SP-2250/SP-2401 column. Without extensive spectral characterization, it cannot be determined which peaks represent the same compounds in the multicomponent formulations.

The chromatograms of the reference standards separations on the OV-330/SP-2250 column are shown as Figure 2. These were recorded on

TABLE 1. TOTAL RESIDUE FROM VARIOUS SLUDGE SAMPLES

Sample	Percent Residue	
	Homogenized and Stirred	Stirred Only
San Antonio Primary	3.59 $\pm$ 0.06	3.64 $\pm$ 0.07
San Antonio Digested	3.81 $\pm$ 0.05	3.79 $\pm$ 0.06
Dayton Digested-A	6.26 $\pm$ 0.00	6.19 $\pm$ 0.06
Dayton Digested-B	5.85 $\pm$ 0.01	5.90 $\pm$ 0.02
Dayton Digested-C	5.58 $\pm$ 0.14	5.89 $\pm$ 0.02
Dayton Combined-A	4.88 $\pm$ 0.03	5.72 $\pm$ 0.08
Dayton Combined-B	6.36 $\pm$ 0.07	6.26 $\pm$ 0.00
Dayton Primary	5.76 $\pm$ 0.35	6.23 $\pm$ 0.03
Dayton Raw	7.79 $\pm$ 0.04	
Cincinnati Primary	6.54 $\pm$ 0.02	
Mill Creek Primary	4.94 $\pm$ 0.12	
Muncie Primary	5.28 $\pm$ 0.08	

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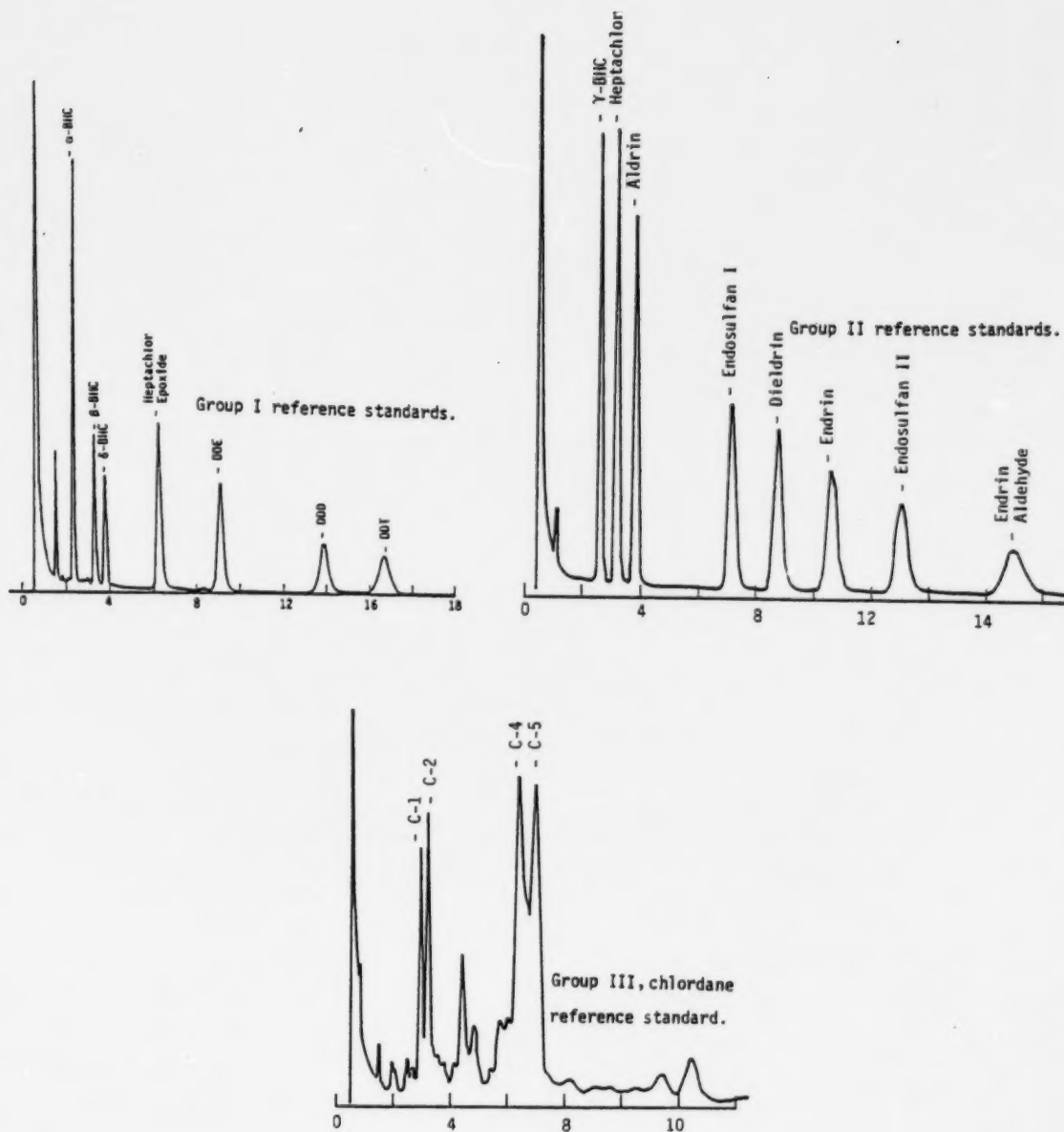


Figure 1. Gas chromatography of chlorinated reference standards on SP-2250/SP-2401 column

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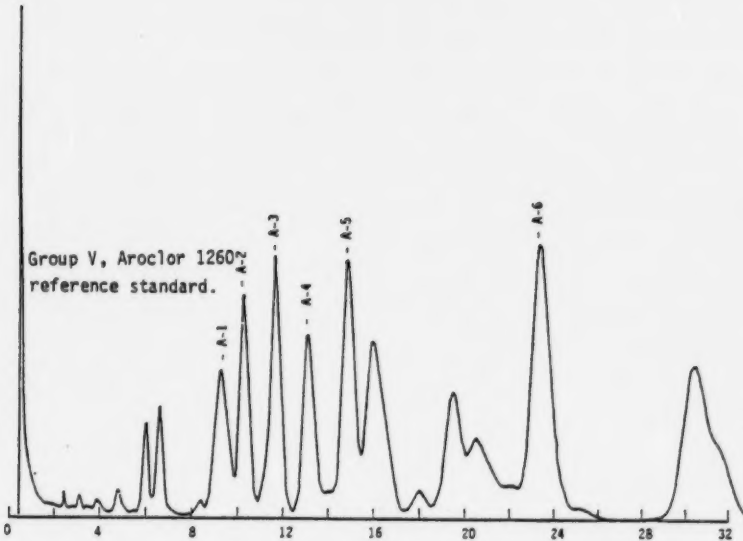
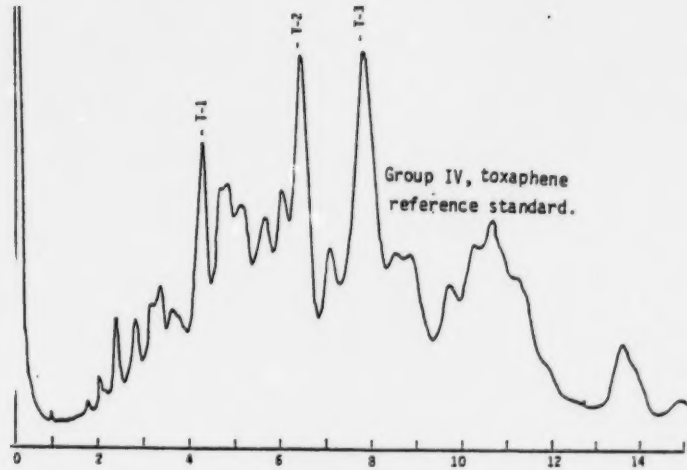


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TABLE 2. RETENTION TIMES OF CHLORINATED PESTICIDES AND AR 1260

Compound	Retention Time*	
	Column I	Column II
α -BHC	0.56	0.58
γ -BHC	0.69	0.77
β -BHC	0.78	1.14
Heptachlor	0.84	0.84
Chlordane (C-2)	0.84	0.86
δ -BHC	0.90	1.42
Aldrin	1.00	1.00
C-3	1.13	1.21
Heptachlor Epoxide	1.46	1.70
C-4	1.62	1.91
C-5	1.75	2.04
Endosulfan I	1.82	2.05
DDE	2.12	2.74
Toxaphene (T-1)	2.20	2.65
Dieldrin	2.21	2.63
Aroclor 1260 (A-1)	2.38	2.02
A-2	2.63	2.86
Endrin	2.66	3.14
A-3	2.98	3.21
C-6	3.12	3.77
DDD	3.20	4.53
Endosulfan II	3.23	4.23
T-2	3.28	3.28
A-4	3.36	3.63
A-5	3.79	4.07
DDT	3.86	4.93
T-4	3.97	4.51
Endrin Aldehyde	5.95	5.16
A-6	5.95	5.88

* Calculated relative to Aldrin for

Column I = 1.5% SP-2250 + 1.95% SP-2401

Column II = 1.7% SP-2250 + 1.7% OV-330 both on 100/120 mesh Supelcoport

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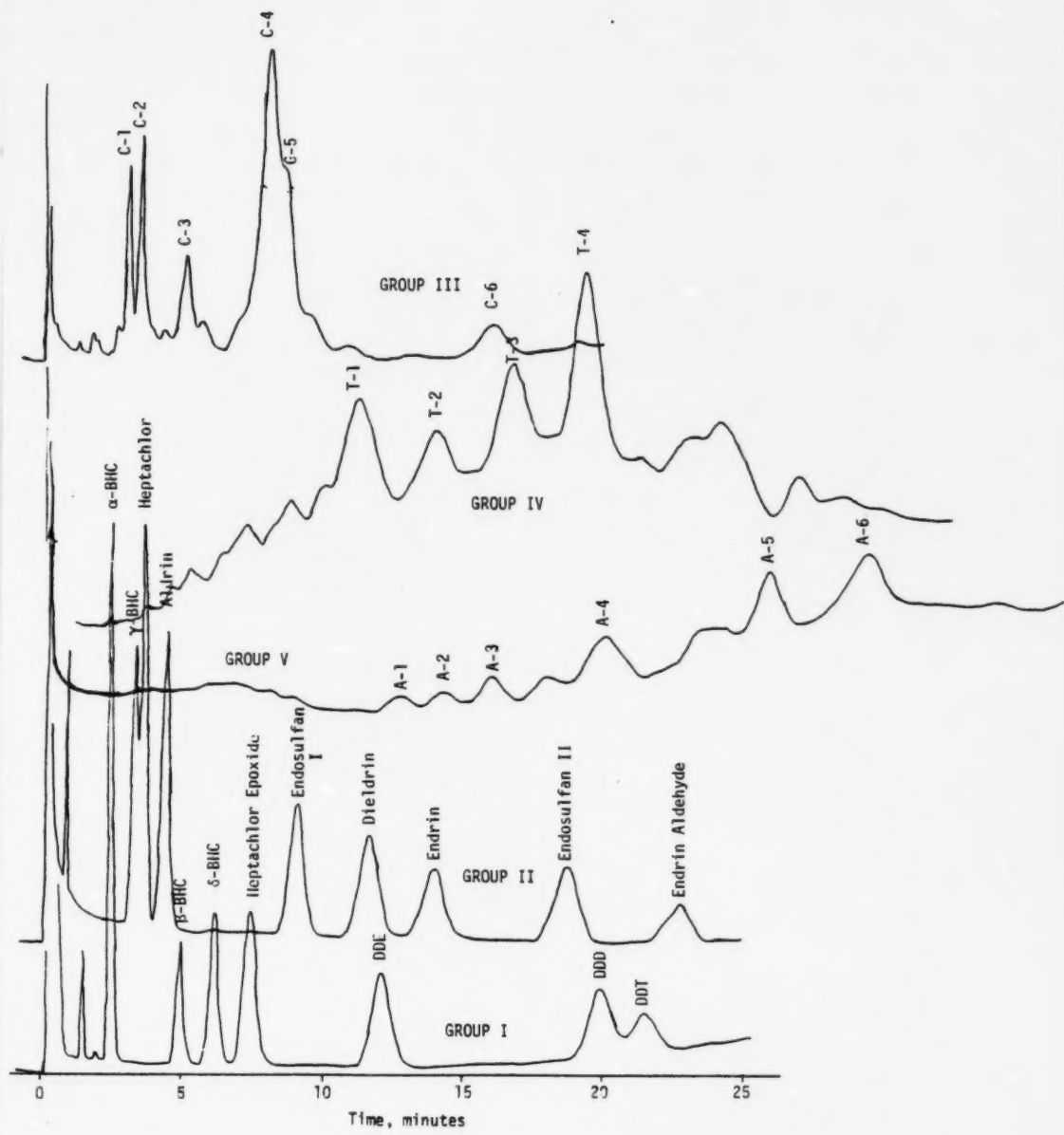


Figure 2. Chromatograms of reference standards separated on OV-330/SP-2250 column

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another brand of instrument and the scales are different which makes it somewhat difficult to make comparisons. Of the multicomponent formulations, the pattern for chlordane is most obvious. It is less so but still discernible for toxaphene and mostly a matter of guessing for Aroclor 1260.

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The liquid phase, OV-330, provides some interesting characteristics for the gas chromatography of the chlorinated pesticides and biphenyls. With further experimentation, it should provide a good backup column for the identification of the organochlorines. Other liquid phases which deserve consideration for this type of application are OV-73, a diphenyldimethyl silicone gum, and OV-210, a trifluoropropyl silicone oil.

We also attempted to further characterize the sludges by gas chromatographing the extracts with an instrument equipped with FID. This was done in the expectation that more information on the nature of the compounds in the various extract fractions would help formulate better isolation and cleanup procedures. Packed columns were found to be totally useless for these separations since the amount of organics in the extracts overwhelmed the FID regardless of the cleanup done. There was so much organic matter the columns could not separate the individual compounds. The chromatograms consisted usually of a small "envelope" in the first few minutes to a larger one which extended beyond 30 minutes of run time. Occasionally, some individual peaks would be superimposed on the "envelopes" of some of the extracts.

Better resolution of the organics in sludge extracts was sought by using a 30-meter, SP-2250 SCOT column. Chromatography on this column also resulted in the "envelope" effect, although the peaks of individual compounds were superimposed in a sawtooth effect. Since it was obvious that characterization of sludge in this manner would require an extensive effect and was intended to be of an exploratory nature, the work was abandoned.

Another approach available for the confirmation analysis of sludge extracts is the use of a Hall electrolytic conductivity detector in the halogen-specific mode. The HECD was expected to provide the most useful information because of its greater specificity over the ECD. Unfortunately, the complexity of the sludge extracts caused this to be a fruitless approach. The very high organic content in the extracts is apparently reacted to conductive species in the reaction tube of the HECD. This results in an extremely high background which could probably be contended with were it constant but it tends to meander between the limits of the response region.

Although the FID and HECD were not successfully applied on this project, it is felt that these detectors can provide useful data. The use of these detectors, other liquid phases and capillary columns all need to be further evaluated for application to the determination of the organochlorine compounds in sludge.

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EXTRACTION METHODS

The results of extraction of San Antonio sludges spiked with known amounts of polychlorinated compounds are shown in Table 3. Sample weight was 20 g and the sample spiked with Group I or Group II contained 1 μ g of each compound, Group III 10 μ g of chlordane, Group IV 50 μ g of toxaphene, and Group V 20 μ g of Aroclor 1260. These recovery values were calculated from peak areas recorded by digital integration techniques with a laboratory computer system interfaced to the gas chromatograph through an electronic analog-to-digital converter. The chromatograms were complex and caused a greater scatter in calculated peak areas because the computer could not recognize the peaks and differentiate among them. Very slight differences in peak characteristics from one run to the next would cause the computer to lose or gain one to three visibly discernible peaks in a peak area measurement.

The chromatograms shown in Figure 3 may be used to point out the problems in computing peak areas. The peak area calculated for Peak 2 in the spiked sludge did, in some cases, include the area of the peak immediately following it in the unspiked sludge. Similarly, the computer recognized Peaks 4 and 5 from the unspiked primary sludge as only one peak and detected two peaks in the other chromatograms. The calculated peak areas might or might not have contained the areas of the peaks preceding or depending on whether the slope sensitivity of the integrator recognized an inflection point. In several cases, these changes were subtle enough that the chromatograms had to be visually inspected to recognize them. Manual measurement of peak heights was considered to provide more reliable calculations. Consequently, all subsequent calculations were based on peak height measurements made manually by hand and rule. Although more tedious and time consuming, it was felt that the result would be more reliable data for the determination of procedural efficiencies.

The results shown in Table 3 on first glance would only rule out the liquid-liquid method on the basis of very low recoveries. But closer evaluation of the data in combination with the visual inspection of the more than 300 chromatograms from which these data were taken led us to choose the centrifuge method of extraction for further study. Other considerations such as the possibility of thermally induced reactions in the Soxhlet and liquid-liquid methods, the limited availability and greater expense of continuous liquid-liquid extractors, and the assessment by laboratory personnel that they had a greater degree of control over the manipulations of the centrifuge method served to reinforce this decision.

Another set of experiments was conducted with San Antonio primary sludge to further test the centrifuge method. Each of the five groups of compounds was spiked into individual 20 g wet sludge samples at two levels, generally 100% and 30% of a nominal level in 20 g wet sludge. The nominal (100%) level was 1 μ g for Groups I and III, 10 μ g for Group II, 50 μ g for Group IV and 20 μ g for Group V. This provides spike levels of 1 and 0.3 μ g, 10 and 3 μ g, 50 and 15 μ g, and 20 and 5 μ g, respectively. For the sake

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TABLE 3. COMPARISON OF EXTRACTION TECHNIQUES FOR ORGANOCHLORINE COMPOUNDS

Method	Sludge + Type	Percent Recovery*													
		Group I							Group II						
		α-BHC	β-BHC	δ-BHC	Hept. Epox.	DDE	DDD	DDT	γ-BHC	Hept.	Aldr.	End. I.	Diel.	End. II	Endr. Ald.
Centrifuge	P	84	80	66	95	81	82	59	90	87	84	98	76	78	96
	D	171	148	122	96	188	60	2	66	79	98	89	129	138	88
Column Elution	P	102	86	75	109	103	102	79	80	99	95	106	98	72	116
	D	216	198	134	86	166	76	15	141	111	169	93	173	147	91
Soxhlet	P	95	100	70	97	86	91	70	85	94	72	106	89	73	114
	D	189	316	199	168	370	107	58	110	146	119	56	100	91	61
Liquid-Liquid	P	54	62	38	47	37	37	35	134	78	86	90	80	75	87
	D	4	0	0	0	2	0	0	54	13	16	17	10	18	18
Group V - Aroclor 1260															
Centrifuge	P	93	96	60	80	30									
	D	75	50	81	86	56									
Column Elution	P	44	66	99	98	44									
	D	90	92	72	74	54									
Soxhlet	P	72	10	76	87	59									
	D	98	107	107	119	82									
Liquid-Liquid	P	44	29	34	43	45									
	D	1	5	14	14	7									

* Average of two determinations for each sludge type and method except liquid-liquid; cl-anup by Florisil column chromatography.

† San Antonio sewage treatment plant; P = primary sludge, D = digested sludge.

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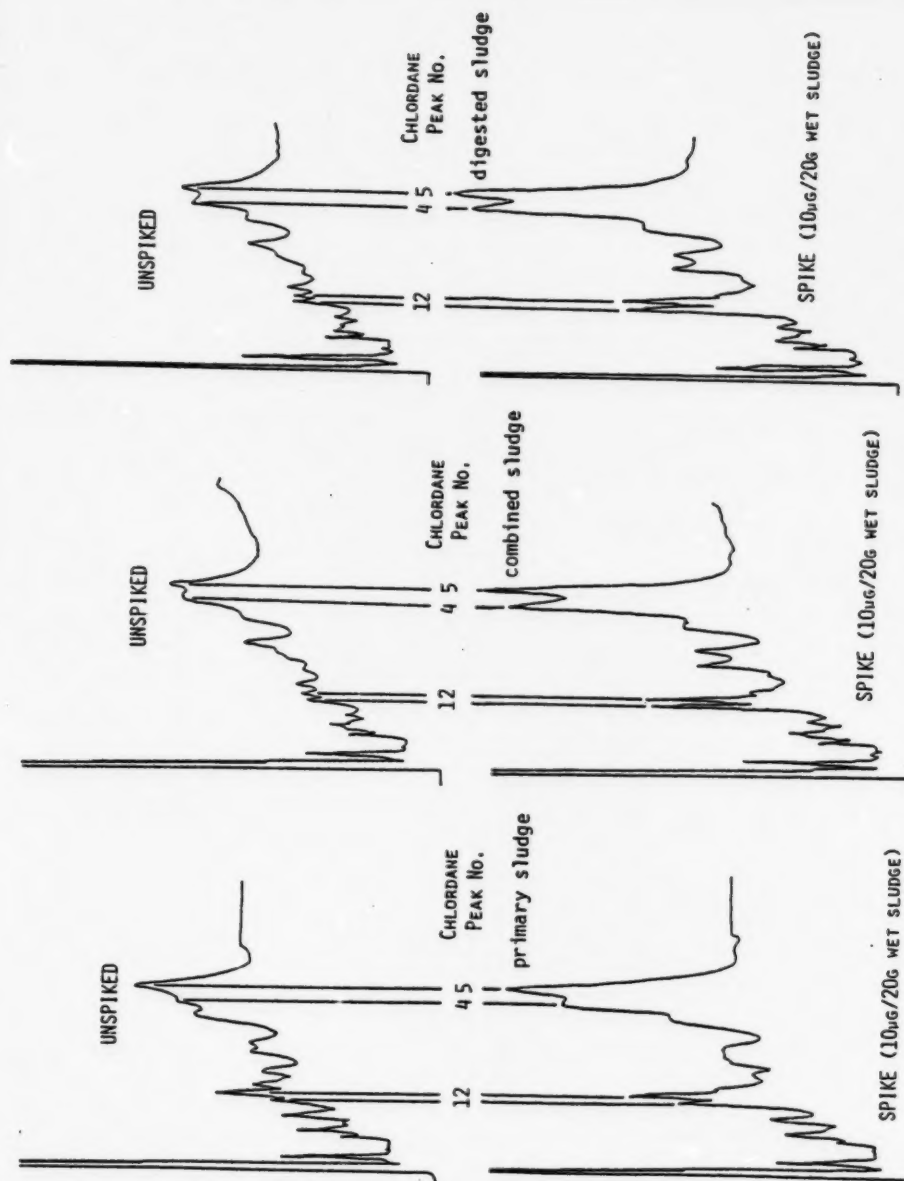


Figure 3. Chromatograms of extracts from spiked and unspiked sludges

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of consistency and simplicity, spiking was done on a wet sludge basis. Normally, this made little difference since solids content in all of the sludges used was fairly consistent within the range of 3.5-6%. These levels were used for all determinations from this point on and will simply be referred to as the 100% and 30% spike level. The spiked samples were allowed to equilibrate for 18 hours prior to extract preparation. This period allowed the spiking compounds to attain a degree of equilibration between the suspended solids and aqueous solution to more closely represent the distribution of endogenous compounds. After equilibration for 18 hours, the samples were extracted by the centrifuge method. The extracts, then, were chromatographed after cleanup, and the recoveries of the individual compounds were calculated from both computer integration and peak height data. Each group determination was accompanied by extraction of unspiked sludge replicates to provide correction of the data by subtraction of "blank" values. The data obtained from these experiments are shown in Table 4 as average percent recovery for each compound.

The peak height data exhibit more reasonable recovery values, especially for Groups I and II. There was good precision between replicates for both the computer integration and peak height measurements, but the latter was more reliable because the analyst could adjust to chromatographic anomalies. This was not always possible with computer integration, even by changing the calculation program parameters.

Recoveries were fairly consistent by the peak height calculations, in the 70 to 90% range for most of the Group I and II compounds at the higher concentration levels. Recoveries for the lower spike levels were noticeably less consistent, especially for the Group II compounds. Groups III, IV and V, the multicomponent formulations, exhibited very inconsistent recoveries for the compounds within each group. For example, recoveries of the five peaks calculated for chlordane ranged from 25 to 90% by the peak height method and 44 to 92% by computer integration.

The centrifuge extraction method was then applied to sludge obtained from Cincinnati and Dayton, Ohio municipal sewage treatment plant facilities. As with the San Antonio sludges, 20 g aliquots were spiked at two levels, generally 100% and 30% of a nominal level. The sludges were extracted, cleaned up and analyzed in a manner identical to the preceding work. Representative chromatograms of the three sludges, both spiked with chlordane and unspiked, are shown as Figure 3. These chromatograms show strong evidence for the presence of the Group III pesticide, chlordane. The four peaks normally measured for chlordane are quite apparent in the unspiked sludges and give the same relative response as the authentic standards. These sludges also exhibited responses for the BHC's, both endosulfans, DDT, heptachlor epoxide, and Aroclor 1260.

Electron capture chromatography of all sludges demonstrated about the same background levels. It is possible the San Antonio sludge showed slightly lower background levels than the others due, in part, to a smaller percentage of wastewater influent from industrial sources. In all types of

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TABLE 4. EXTRACTION EFFICIENCY OF SINGLE COMPONENT PESTICIDES

Sludge Type	Spiking Level	Average % Recovery*													
		Group I							Group II						
		α-BHC	β-BHC	δ-BHC	Heptachlor Epoxide	DDE	DDD	DDT	γ-BHC	Heptachlor Aldrin	Endosulfan I	Dieldrin	Endrin	Endosulfan II	Endrin Aldehyde
Cincinnati Primary	0 [†]	0	0.30	0.28	0.24	0.12	0	0.70	0.19	23	0.15	0.56	0.19	0.57	0
	30	88	37	66	91	71	50	75	0	45	30	0	37	0	0
	100	83	48	60	73	62	59	53	57	68	55	31	71	61	22
Dayton Combined	0 [†]	0.04	0.19	0.12	0.12	0.03	0	0.34	0	0.07	0.02	0.16	0.02	0.25	0
	30	83	56	59	61	53	38	75	79	62	48	38	93	70	0
	100	90	84	74	80	68	59	74	93	93	71	59	89	89	15
Dayton Digested	0 [†]	0.03	0.26	0.18	0.15	0.04	0	0.40	0.04	0.06	0.04	0.26	0	0.21	0
	30	93	63	73	97	76	67	113	51	48	37	6	73	54	0
	100	104	107	116	85	71	70	91	90	71	58	48	79	101	98
		GROUP III-Chlordane													
		GROUP IV-Toxaphene													
		GROUP V-Aroclor 1260													
Cincinnati Primary	0 [†]	3.8	2.9	4.1	0	6.5	12	4.2	4.9	6.7	2.3				
	30	77	91	107	86	48	0	0	0	0	41				
	100	62	110	35	103	106	78	88	89	96	95				
Dayton Combined	0 [†]	1.8	3.6	3.9	3.1	0	5.2	2.6	0	3.7	2.3				
	30	52	124	68	85	57	75	81	45	43	85				
	100	55	33	41	72	67	84	37	33	30	34				
Dayton Digested	0 [†]	1.7	3.8	4.1	0	0	2.9	0	0	2.9	1.1				
	30	52	51	29	56	29	85	107	50	51	73				
	100	41	48	45	115	103	55	64	45	42	63				

* Cleanup by Florisil column chromatography.
[†] ng apparently present in unspiked sludge, "blank" value.

sludges, difficulty was encountered in trying to use computer integration for the determination of peak areas necessitating peak height measurement for all data obtained. The percent recoveries obtained using the three sludges from different locations are summarized in Table 4.

The recoveries for Group I, the recoveries were reasonably close to those from the San Antonio sludge for α -BHC and DDE. The percent recovery of α -BHC was high for all sludges, indicating at least 80% can be expected down to the 0.3 $\mu\text{g}/20\text{ g}$ level, and recoveries above 90% occurred frequently. The results for the combined sludge were slightly lower for DDE, but were acceptable at the higher spike level. The recovery of DDT from the sludges studied here was significantly higher than obtained in most cases from the San Antonio sludge, and recovery of heptachlor epoxide was roughly equivalent. The remaining compounds gave quite variable results depending upon the spike level and the matrix.

The most significant result for Group II was the low recoveries obtained using the low level spike on the primary sludge. Only four of the eight compounds were recovered at all, and these were quite low. In general, much better recoveries were observed for the 100% spike versus the 30% spike, similar to those seen in the San Antonio sludge. Endrin aldehyde was only infrequently recovered in the three sludges, compared with the 45-47% recovery consistently obtained from the San Antonio sludge.

Recoveries for the 100% spike were acceptable, in general, with endosulfan I being the principal exception. At the 100% spike level, the highest recovery was 58.7% for the combined sludge, and ranged to 31.4% for the primary sludge. In contrast, recoveries of 98 and 92%, respectively, were obtained at the 30% and 100% levels using the method on San Antonio primary sludge. Endosulfan II was not recovered well from the combined sludge in comparison to the remaining matrices. This appears not to be related to the background level observed.

Comparative chlordane recoveries are made using only numbered peaks, 2, 4, and 5. In the San Antonio sludge, quantitation was feasible on Peaks 2 through 5, while on the remainder, only Peaks 1, 2, 4, and 5 were usable, and the comparison is made only on those peaks in common. This points out an additional problem area, as it may be difficult to determine which of the compounds will persist in any given matrix and therefore to define a chlordane result.

Less complete recovery was observed in general for the multicomponent formulations of Groups III, IV and V than for the previous effort using the San Antonio sludge. In all four matrices, however, the recoveries for the various peaks were inconsistent; no pattern existed where one or more of the studied peaks gave the highest recovery in all matrices. This further complicates the quantification of the multicomponent substances.

For toxaphene in Group IV, the individual peak recoveries were as inconsistent as they were in the San Antonio sludges, although the

recoveries were higher and more consistent for the higher spike level. No one peak could be singled out as indicative of the true concentration of toxaphene and it would require a much more extensive study to determine which peaks, if any, should be used to quantify toxaphene.

The results for Group V (AR 1260) were similar to those for chlordane in that not all peaks were quantifiable and there was generally no consistency among the peaks as to the measured recovery. Though essentially no recovery of AR 1260 existed in the primary sludge at the low spike level, good recovery was charted at the 100% level. The most consistent results were obtained on the combined sludge at the high level, but the recovery was only around 30-35%. As in most other groups, the overall recoveries obtained were neither as high nor as consistent as those from the previously studied San Antonio sludge.

Later in the program some brief experiments were conducted in an attempt to determine the effects of sample pH on extraction efficiencies of the various organochlorine compounds. This provided some preliminary data for including the chlorinates in an integrated method for the analytical determination of the organic priority pollutants in municipal sewage treatment plant sludge. Two sludges were used, Dayton combined and Cincinnati primary, and were spiked at the 30% and 100% levels with Groups II and V. These two groups were chosen to be representative of the five groups of organochlorine compounds.

The spiked sludges were made either alkaline or acidic prior to extraction in the usual manner alongside unspiked sludges. The unspiked sludge responses on the gas chromatograph were used to correct the spiked levels by subtraction of "background" responses. The extracts were cleaned up by gel permeation chromatography on Bio-Beads S-X2 using DCM as the mobile phase. By this time, gel permeation chromatography had replaced Florisil fractionation as the preferred cleanup procedure. The results of this study are shown in Table 5 and are calculated as the average percent recovery from duplicate samples of the known spike levels.

At this stage in the program, laboratory personnel had become more proficient in handling the sludge due to the many samples that had been analyzed. This was reflected in the more consistent results obtained from the analysis. Most of the recoveries from spiked sludges were above 60% for the 30% spike level except for compounds such as DDT, endosulfan II, and endrin aldehyde. Over the period of this program, these three compounds resulted in the most inconsistent recoveries by all methods tried.

The most obvious of the results is the recovery of endrin aldehyde from acidic sludge. The results overall are almost inconclusive. There is apparently a tendency toward higher recoveries from the alkaline sludge. This tendency is more obvious for the PCB formulation than for the pesticides. In comparison with the results from Table 4, these values reflect a marked improvement in recoveries. This may be, however,

TABLE 5. RECOVERY OF ORGANOCHLORINE COMPOUNDS BY EXTRACTION FROM ALKALINE AND ACIDIC SLUDGE

Average Percent Recovery*									
Sample	Spike Level	γ -BHC	Heptachlor	Aldrin	Endosulfan I	Dieldrin	Endrin	Endosulfan II	Endrin Aldehyde
GROUP II PESTICIDES - Sample pH = 10									
Dayton	0	0	0.07	0	0	0.01	0	0	0
Combined	30	73	73	73	107	83	107	90	0
	100	100	91	87	98	108	121	105	3
Cincinnati	0	0	0.26	0	0	0	0.48	0.40	0
Primary	30	80	77	100	207 [†]	110	67	50	0
	100	88	80	86	86	93	76	63	0
GROUP II PESTICIDES - Sample pH = 2									
Dayton	0	0	0.10	0	0	0.02	0	0	0
Combined	30	73	60	63	113	73	92	83	60
	100	91	86	76	90	99	109	97	77
Cincinnati	0	0	0.29	0	0	0	0.55	0.39	0
Primary	30	87	83	100	197 [†]	57	57	47	70
	100	93	91	90	91	72	74	76	73

	A-1	A-2	A-3	A-4	A-5	A-6			
GROUP V AROCLOR 1260 - Sample pH = 10									
Dayton	0	0	4.3	1.4	0.9	2.6	0.8		
Combined	30	92	57	75	83	78	83		
	100	73	64	68	70	75	73		
Cincinnati	0	13.5	11.0	3.9	7.6	7.1	1.1		
Primary	30	50	79	80	79	89	88		
	100	13	75	73	50	95	83		
GROUP V AROCLOR 1260 - Sample pH = 2									
Dayton	0	0	2.7	1.3	0.6	1.8	0.7		
Combined	30	75	50	57	63	53	60		
	100	75	72	73	73	74	74		
Cincinnati	0	11.4	10.7	3.7	6.0	6.7	0.9		
Primary	30	28	43	.53	65	60	68		
	100	33	48	54	55	55	61		

* ng apparently present in unspiked 20 g sludge, "blank" value.

† background peak interferes with low level spike determination.

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attributed in large part to the experience of the laboratory personnel mentioned previously.

The preliminary results of the acid/base extractions indicate that with some more experimentation, the incorporation of the determination of the chlorinated pesticides and biphenyls into an integrated organic priority pollutants method should be quite possible. These compounds are going to be extracted from either acidic or basic sludge after which several options are available. The detection of chlorinated compounds by electron capture is inherently more sensitive than the other organic priority pollutants. This makes it possible to use a smaller portion of the extract containing these compounds. The total extract may then either be subjected to further cleanup, or a small portion may be split off for organochlorine determinations. Alternately the acid or basic components may be separated by an aqueous back extraction which in the process would provide another cleanup. Such a separation scheme would have the added advantage of producing additional information for the identification of the compounds in question.

CLEANUP PROCEDURES

The method most widely applied to the cleanup of pesticides has been chromatographic fractionation on Florisil. Florisil cleanup has been used extensively in this laboratory in a modified form. The procedure used in this laboratory calls for reducing the activity of the Florisil by the addition of 5% water and collecting two fractions, with 6% and 50% diethyl ether in hexane, respectively.

Duplicate extracts were made in the usual manner of sludges spiked with Groups I, IV, and V and fractionated on activated Florisil-PR and 5% water-deactivated Florisil-PR. The fractions were then analyzed by GC/ECD. Inspection of the average recoveries from the two methods shown in Table 6 readily indicates there is no great difference between the two methods. The only differences noted were qualitative during the elution. The colors observed in the water-deactivated Florisil-PR column were more pale and extended almost all the way down the column, whereas the colors were darker, more distinct and extended only halfway down the activated Florisil-PR column.

In an effort to determine how much material was being removed by Florisil cleanups, extracts of several unspiked sludges were fractionated on Florisil columns, the fractions were evaporated to dryness, and the residues were weighed. The total extractable residue was determined for triplicate samples and averaged. The fractionations were then done on three additional samples which had been carefully weighed to 20 g and the extracts had not been taken to dryness. The material in each fraction was calculated as a percent of the total extractable residue which had been determined separately. The dried residues were also chromatographed on Florisil. The difference between total recovered and total extractable residue was the amount removed by the Florisil column. The results of this

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TABLE 6. COMPARISON OF CLEANUP OF TWO FLORISIL ACTIVITY GRADES

Sorbent	Average % Recovery														
	Group I							Group IV			Group V				
	α -BHC	β -BHC	δ -BHC	Hept. Epox.	DDE	DDD	DDT	T-1	T-2	A-1	A-2	A-3	A-4	A-5	A-6
Deactivated Florisil	72	83	81	75	59	56	119	72	60	40	56	48	36	48	56
Activated Florisil	70	77	78	71	57	51	118	68	58	47	63	54	40	54	63

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experiment shown in Table 7 indicate that the amount of residue material removed from the organic extractables ranged from 9 to 47% excluding the one questionable result for the Mill Creek primary sludge. There are two sets of values for each sludge sample; the upper series of numbers is for the extract that was not taken to dryness, and the lower series is the fractionation done on the residue which had been dried and weighed. The residue recovered from the column is eluted in the two fractions retained for analysis. The larger part--an average of 61% ranging from 45 to 82%--is eluted in the less polar 6% ether fraction which contains 15 of the 17 polychlorinated formulations. The cleanup effect is for the most part a change in the distribution of analytes and interferences. Distributing these substances into two fractions effectively reduces the background in relation to the analytes and aids in removing some background by deposition on the column as well. The interfering compounds apparently are similar enough in their properties to the chlorinated compounds in that they effectively resist separation based on the adsorption and partitioning effects of the Florisil chromatographic system. Apparently, drying the residue prior to Florisil chromatography has no effect on this particular determination. There is no difference in the distribution of the fractions when compared to those from extracts which had not been dried prior to cleanup on Florisil.

Many of the interfering compounds are postulated to be hydrocarbons of higher molecular weight, such as paraffins, lipids, oils, and greases. These compounds should, for the most part, be of significantly greater molecular size than the polychlorinated hydrocarbons, and hence, should be amenable to separation by size exclusion chromatography. As will be discussed later, the Florisil cleaned up extracts proved to contain too much interfering material, and as a result, gas chromatographic/mass spectrometric determinations could not be made. The use of gel permeation materials was then explored in an attempt to provide cleaner extracts for GC/MS analyses.

Sephadex LH-20, a dextran gel, was used in the first system evaluated. Cyclohexane was used as the mobile phase. Sephadex LH-20 has strong adsorptive properties in addition to the size exclusion characteristics, especially during the chromatography of aromatic and polycyclic compounds. Initial experiments with polychlorinated standard mixtures resulted in recoveries of less than 40% for most of the compounds. Some were completely lost, apparently adsorbed on the column, requiring a more polar solvent to elute them.

Bio-Beads S is a series of porous styrene-divinyl benzene copolymer beads used as a gel permeation material which is much less adsorptive than Sephadex LH-20. Bio-Beads S-X2 gel, which was used for the initial GPC determinations, has a molecular weight operating range from 100 to an upper limit of 2700, the molecular weight exclusion limit. Cyclohexane was used as the mobile phase and the polychlorinated compounds were found to elute in Fractions 2 through 6 when six 25 mL fractions were collected. The multicomponent groups tended to elute in broad bands over two or in some cases three fractions.

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TABLE 7. DISTRIBUTION OF EXTRACTS IN CLEANUP FRACTIONS

Sample	Extract Amount mg	Florisil Column			Extract Amount mg	Bio-Beads S-X2 Column		
		Extract Fraction Amount	Fraction 6% Ether	Fraction 50% Ether		Fraction Discard	Fraction II Chloros	Fraction Removed
San Antonio Digested	49	64.4 45.3	10.5 7.4	25.1 47.3	60	85.1 78.8	25.4 21.8	-10.5 - 0.6
San Antonio Primary	61	55.2 Accidental sample loss	13.6	31.2	67	76.3 74.0	11.3 13.4	12.4 12.6
Mill Creek Primary	101	59.7 123.9	10.1 13.6	30.2 -34.7	105	69.1 75.2	19.1 20.7	11.8 4.1
Dayton Raw	195	62.3 61.7	7.5 8.5	30.2 29.8	190	71.9 78.7	25.4 15.5	2.7 5.8
Dayton Digested	128	81.9 80.6	8.7 8.4	9.4 11.0	124	68.9 66.7	32.4 30.3	- 1.3 3.0
Cincinnati Primary	143	68.5 69.2	7.3 9.5	24.2 21.3	138	80.5 72.8	23.0 29.8	- 3.5 - 2.6

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The commercial literature included with the S-X2 beads suggested that a more active solvent would elute the polychlorinated compounds in a narrower band. The liquid phase was changed to DCM since it is not only more active than cyclohexane but it is more volatile and in it, solutions be concentrated more readily. An additional advantage was the fact it is used extensively in organic pollutants analyses.

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Standard solutions of the five groups were chromatographed on the S-X2. The Group I and II separations had 1 μ g of each compound placed on the column, and for the others there was Group III, 10 μ g; Group IV, 50 μ g; Group V, 20 μ g. Initial studies indicated much of the background interference was eluted in the first 85-90 mL DCM coming off the column and the pesticides/PCB's were in the next 25 mL. Accordingly, the first 85 mL eluted from the columns was discarded, the next 5 mL was caught as Fraction 1 and the next 25 mL as Fraction 2.

Each fraction was then concentrated and chromatographed. The percent recovery was calculated for each peak used for these determinations, based on the known amount of reference material placed on the column. Recoveries were over 80% except for δ -BHC (47%), γ -BHC (75%) and endrin aldehyde (69%). The amounts detected in Fraction 1 were negligible except for heptachlor epoxide (7%), DDD (3%), and endrin aldehyde (4%). Considering the broad range of compounds, these results are adequate and quite acceptable. The results are also apparently repeatable with good precision but some more extensive experiments would have to be conducted to determine the statistics of this process.

In order to compare efficiencies of the cleanup methods for removing interferences, four sludges were extracted in the usual manner. The extracts were taken to dryness under a stream of pure, dry inert gas and weight of extractable organics was determined. The residues were then taken up in DCM and transferred to gel permeation columns and eluted with DCM. Duplicate separations were carried out on extracts which had not been taken to dryness prior to being placed on the gel column. The first 90 mL which normally would be discarded was caught and retained as Fraction 1 and the next 50 mL which normally would contain the pesticides and PCB's was retained as Fraction 2. The two fractions were evaporated to dryness and residue weights determined. The percentage recovery was calculated for each fraction and the results of these determinations are compiled in Table 7 where they are compared with results of the Florisil cleanup fractionations.

Gel permeation chromatography removes an average 75% of unwanted materials from the sludge extracts. This ranges from 67% for the Dayton digested sludge to over 80% for the San Antonio sludge with no discernible pattern. The amount of material which can be removed by GPC will probably vary on a continuing basis at each plant as a function of influent. Florisil removes about a third as much as the gel column, again, with no apparent correlation as regards the type of sludge. What this means in terms of the determinative step in the analytical method is that in some

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cases, when 1 ng of a pesticide or PCB component is injected into the gas chromatograph, about 50,000 ng of other substances are injected along with it if it had been cleaned up on a gel column. For this same sludge extract cleaned up by Florisil fractionation, the ratio would be 1 ng analyte to about 100,000 ng "background," which is an increase in "background" by a factor of two. Apparently gel permeation chromatography provides a better cleanup than does Florisil chromatography. There is still, however, a significant amount of material that could be removed to enhance these analyses.

The recoveries of compounds from GPC discussed previously make this an attractive method for the cleanup of sludge extracts. The losses of the organochlorines were cause for concern though, because any compounds being adsorbed on the gel would be subject to breakthrough at any time and would affect results of later determinations. The only other place these compounds could have been lost was through volatilization during evaporation of the solvent. In an attempt to learn where these losses were occurring, 1 mL of spiking solutions containing Group I and Group V were added separately to duplicate 75 mL portions of DCM. The solutions were then carried through all the solvent evaporation steps that would normally occur in an analytical sequence and which are listed below.

1. Evaporate in a Kuderna-Danish apparatus.
2. Take down to 2-3 mL under a stream of pure, dry inert gas.
3. Rinse into another Kuderna-Danish apparatus with DCM to a volume of 25 mL DCM, add 5-10 mL hexane.
4. Evaporate as usual, add 100 mL hexane.
5. Evaporate and transfer to a vial.
6. Dilute to 10 mL and analyze as usual.

The recoveries of each component in these solutions were calculated in the usual manner.

The recovery of the seven compounds in Group I and six peaks measured for Group V ranged from 75 to 86%, except for δ -BHC, which was recovered at 39%. The pattern of recoveries very closely matched the pattern noted for recovery from GPC. Additional experiments with Groups II, III and IV confirmed these observations; recoveries ranged from 72 to 90% with γ -BHC (72%) and endrin aldehyde (64%) again coming in on the low end.

The comparison of these data with those from the GPC recovery studies suggests that most, if not all, these losses can be attributed to the evaporative processes causing volatilization of the pesticides and PCB's. These losses are probably diminished somewhat in a sludge extract because the high boiling substances act as a "keeper" that serves to decrease volatilization losses of the polychlorinateds. Loss of the compound might then proceed through some other mechanism such as adsorption or reaction.

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Florisil cleanup was replaced by GPC on Bio-Beads S-X2 with DCM as the mobile phase. Bio-Beads S-X3, a gel in the same class as S-X2 but with a molecular weight exclusion limit of 2000 and a molecular weight operating range up to 2000 was also evaluated for possible use as a cleanup medium. The elution characteristics of cyclohexane as the mobile phase for either of these gels were also determined. It had been noticed that cyclohexane widens the elution patterns of the chlorinateds and this could be used to prepare fractions amenable to gas chromatography/mass spectrometry.

All five groups of standards were chromatographed on the two gels, each with DCM as the mobile phase and then the experiments were repeated with cyclohexane. For each determination, 10 mL fractions were caught from 75-250 mL elution volume. The fractions were then chromatographed and each peak responding on the ECD was calculated as a percent of known amount of standard placed on the column. The elution profiles plotted as elution volumes versus detector response are shown as Figure 4. For Groups I and II, the responses of α - and δ -BHC, respectively, are plotted as being representative of the two groups. With some minor variations, the elution profiles were identical for any of the compounds within a group. There was no fractionation noted among the peaks of the multicomponent formulations so the response of the various peaks, normally used to measure a group, was averaged within each eluant fraction.

DCM elutes all of the compounds in a volume of 50 mL between 80 and 150 mL, whereas cyclohexane spreads out the elution between 75 to 250 mL. It is these characteristics that make the DCM elution preferred for GC/ECD as it provides maximum cleanup in a relatively short period of time. The cyclohexane elution is preferred for enhancing the response of the analytes relative to background for determinations by GC/MS.

Another series of experiments, much like the one conducted earlier in the program, was conducted to determine efficiency of the overall analytical method. A Tekmar Tissuemizer® homogenizing apparatus became available at this time and replaced manual shaking to mix the sludge and extracting solvent. The homogenizer probe was inserted in the centrifuge tube and the sludge and solvent were mixed at 60% on the homogenizer speed control for one minute. The probe was then raised above the extraction mixture and washed off with hexane applied from a Teflon squirt bottle.

The extracts were cleaned up by GPC on Bio-Beads S-X2 with DCM followed by removal of elemental sulfur by precipitation with metallic mercury. The extracts were analyzed by electron capture gas chromatography. After subtracting the "blank" response of the unspiked sludge, percent recoveries were calculated for each component of the pesticides in triplicate. A summary of the results is shown in Table 8. Overall, these results strengthen the qualification of this method for screening of municipal sewage treatment plant sludges. Compared with the results of a similar set of experiments (Table 14, Interim Report, March 1980), the outstanding feature is the consistency of the results. There is a slight tendency toward better recoveries at the lower spike levels, which

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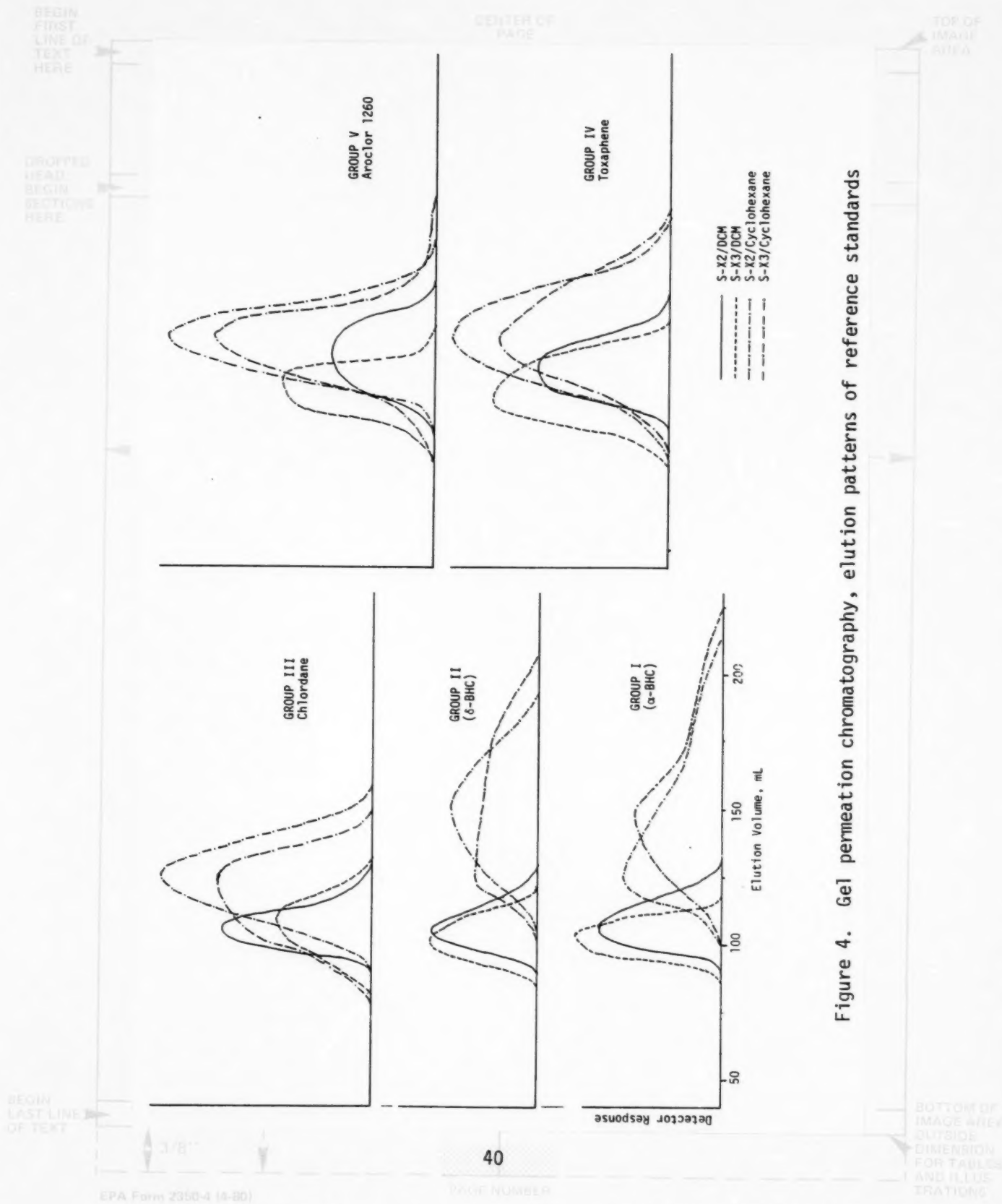


Figure 4. Gel permeation chromatography, elution patterns of reference standards

TABLE 8. RECOVERY OF REFERENCE COMPOUNDS FROM SPIKED CINCINNATI PRIMARY SLUDGE

Spiking Level	Average Percent Recoveries* $\pm$ Standard Deviation						
	GROUP I						
	α -BHC	β -BHC	δ -BHC	Heptachlor Epoxide	DDE	DDD	DDT
0	0.04 $\pm$ 0.01	0.27 $\pm$ 0.05	0.28 $\pm$ 0.04	0.19 $\pm$ 0.04	0.02 $\pm$ 0.02	0.08 $\pm$ 0.03	0.14 $\pm$ 0.03
30	93 $\pm$ 12	69 $\pm$ 32	70 $\pm$ 26	87 $\pm$ 26	86 $\pm$ 14	93 $\pm$ 19	95 $\pm$ 27
100	93 $\pm$ 4	79 $\pm$ 5	81 $\pm$ 8	88 $\pm$ 5	88 $\pm$ 8	99 $\pm$ 13	96 $\pm$ 19

Spiking Level	GROUP II						
	GROUP II						
	γ -BHC	Heptachlor	Aldrin	Endosulfan I	Dieldrin	Endrin	Endosulfan II Aldehyde
0	0	0.01 $\pm$ 0	0	0	0	0	0
30	71 $\pm$ 6	77 $\pm$ 14	73 $\pm$ 4	121 $\pm$ 27	84 $\pm$ 12	62 $\pm$ 17	84 $\pm$ 32
100	68 $\pm$ 4	79 $\pm$ 5	77 $\pm$ 6	74 $\pm$ 8	89 $\pm$ 12	75 $\pm$ 12	88 $\pm$ 16

Spiking Level	GROUP III-Chlordane						
	GROUP III-Chlordane						
	C-1	C-2	C-4	C-5	T-1	T-2	T-3
0	3.4 $\pm$ 0.3	3.0 $\pm$ 0.2	4.4 $\pm$ 0.5	4.7 $\pm$ 0.8	2.2 $\pm$ 0.3	1.9 $\pm$ 0.3	0
30	77 $\pm$ 11	72 $\pm$ 8	92 $\pm$ 21	84 $\pm$ 16	89 $\pm$ 28	89 $\pm$ 19	93 $\pm$ 22
100	81 $\pm$ 9	74 $\pm$ 8	93 $\pm$ 16	86 $\pm$ 9	92 $\pm$ 27	84 $\pm$ 2	91 $\pm$ 7

Spiking Level	GROUP V-Aroclor 1260						
	GROUP V-Aroclor 1260						
	A-1	A-2	A-3	A-4	A-5	A-6	
0	2.1 $\pm$ 0.4	2.3 $\pm$ 0.5	1.8 $\pm$ 0.4	1.1 $\pm$ 0.5	2.7 $\pm$ 0.6	1.3 $\pm$ 0.4	
30	77 $\pm$ 15	80 $\pm$ 7	84 $\pm$ 11	85 $\pm$ 12	88 $\pm$ 2	84 $\pm$ 12	
100	81 $\pm$ 18	84 $\pm$ 15	73 $\pm$ 14	87 $\pm$ 7	89 $\pm$ 13	97 $\pm$ 18	

* Unspiked recoveries in nanograms (ng); DCM eluting solvent.

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are more consistent with the recoveries at the higher spike levels. There is also an apparent tightening of the standard deviations of the recovery values. The greater reliability noted in these results may be attributed to the experience laboratory personnel had gained in doing these particular determinations at this stage in the project. Furthermore, the overall method was more standardized by this time and could be performed on a more routine basis.

At this time, we had a Brinkman Polytron® homogenizer available on a short-term loan basis and we conducted a few experiments in an attempt to determine if the mixing instrument would have any effect on these determinations. Two San Antonio sludges were spiked at the 100% level with the Group I pesticides and extracted in duplicate with each of the two homogenizers. The extracts were cleaned up and analyzed simultaneously and the results calculated as percent recovery. Table 9 shows that the recoveries were essentially identical for the two homogenizers. It was noted that the Polytron appears to be a more durable instrument because it does not have a sleeve bearing in the rotor subject to wear such as the one in the Tissuemizer. We did not have the Polytron on hand long enough to test out this contention.

Another observation, made upon comparison of these results and those in Table, 8 is the different recoveries noted for compounds such as α -BHC, δ -BHC and DDT. This was apparently caused by the presence of background peaks being present in only some of the sludges. There was apparently a synergistic effect in these responses of the spiked sludges on the ECD chromatograms. The nature of these responses would have to be studied more thoroughly by GC/MS to determine how to contend with the problem.

Size exclusion chromatography on Bio-Beads proved an excellent means of cleaning up sludges for quantitative determinations by GC/ECD. Although qualitatively, the chromatograms do not appear much different from those of extracts cleaned up by Florisil column chromatography, gravimetric determinations proved the Bio-Beads removed a great deal more material. Because of their high boiling points, the substances not removed by Florisil would be expected to have been deposited on the gas chromatographic column and thus to cause performance to deteriorate steadily and decrease column life. Therefore, cleanup on Bio-Beads is the recommended approach to quantitative determination of polychlorinated pesticides and biphenyls in municipal sludges.

The removal of elemental sulfur from marine sediment extracts has been practiced successfully in this laboratory for quite some time by the simple addition of metallic mercury to the organic extracts. Due to the potential hazards involved in the continual handling of mercury, two other methods of removing elemental sulfur were briefly investigated. The methods involved contact of the organic solution with copper metal activated by washing with hydrochloric acid or extraction with a solution of tetrabutylammonium sulfite which converts the elemental sulfur to soluble thiosulfate. The activated copper caused a reduction in recoveries of a majority of the

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TABLE 9. COMPARISON OF HOMOGENIZER EXTRACTION EFFICIENCIES

Sample	Instrument	Average % Recovery				
		α -BHC	β -BHC	δ -BHC	Heptachlor Epoxide	DDT
San Antonio Digested	Tissuemizer Polytron	84	92	54	88	57
		82	96	52	85	38
San Antonio Primary	Tissuemizer Polytron	110	86	64	82	92
		122	91	76	88	95

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reference compounds and this approach was quickly abandoned. The tetrabutylammonium sulfite was more effective in removing sulfur although it caused the endrin aldehyde, which in any event is poorly recovered, to be lost completely. Unfortunately, this method introduces several manipulations into an already complex analytical sequence. As a result, the simplicity of the mercury removal method makes it the favorable alternative. Experienced laboratory personnel are required for pesticides analyses, and as such, should be thoroughly familiar with the precautions necessary for the safe handling of mercury.

CONFIRMATION OF IDENTITIES

The confirmation of the identity of polychlorinated pesticides and biphenyls extracted from municipal treatment plant sludges can at best be considered in the preliminary phases of study. Even with the sophisticated computer data handling techniques now available, the complexity and the variety of extractable organic compounds in sludge make the isolation of molecular information a formidable task.

Analysis by GC/MS was performed on an extract of Dayton digested sludge spiked with the Group I compounds at the 30% level of 0.3 μ g of each compound in 20 g wet sludge. The extract had been cleaned up by Florisil fractionation and GPC on S-X2 with cyclohexane eluant. The total ion chromatogram (TIC) of Fraction 3 is shown in Figure 5. The elution points for the seven compounds in Group I are shown on the chromatogram. Although it is known that these compounds were spiked, there is no unequivocal retention time match for any of them.

Single ion chromatograms (SIC) were made at m/z values characteristic of the individual compounds. The reconstructed single ion chromatogram (RSIC) of m/z 183 is shown above the TIC of Figure 5. This ion, in combination with those at 109 and 181, is characteristic of the BHC compounds. RSIC's at m/e 109 and 181 confirmed that only the α -isomer is present in fraction 3; the β - and δ -isomers were found in Fractions 5 and 6 by this technique.

The RSIC's at m/e 246, 248 and 176 indicated the presence of DDE at Spectrum 300. At this point, the mass spectrum, shown in Figure 6, was extracted and cleaned up by the subtraction of appropriate background spectra. The resulting spectrum was then compared to that of an authentic standard shown in Figure 6 above the spectrum of the unknown. Although there is still some contribution from background spectra, the match of ion clusters at m/e 176, 246, and 318 is strong evidence to consider identifying this compound as p,p'-DDE. Using this procedure for each compound in all five groups, confirmation was obtained for each. The multicomponent formulations, however, present special problems due to the many overlapping compounds. As a result, confirmation is difficult, but is possible at a level of 30% of nominal value concentration.

The unspiked digested sludge was searched for the various compounds in the five groups by generating RSIC's of the various characteristic ions.

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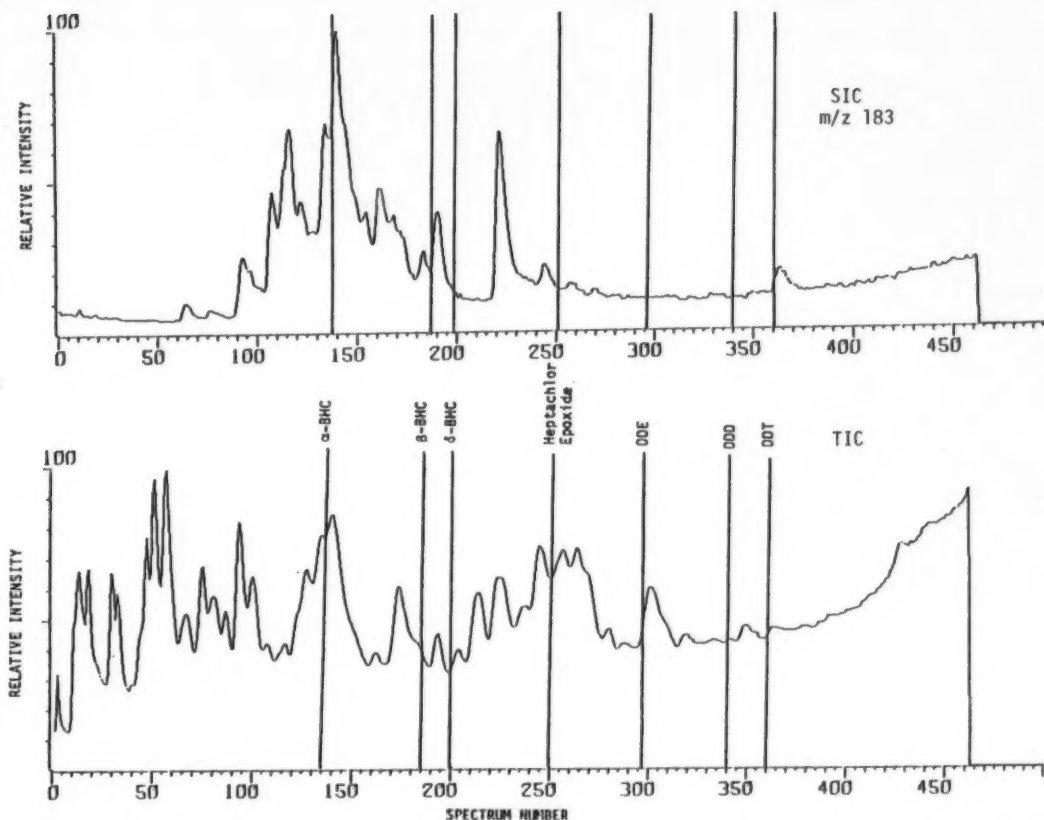


Figure 5. Ion chromatograms of GPC Fraction 3 from organic extract of Dayton digested sludge spiked with Group I polychlorinated pesticides

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This suggested the possible presence of several of these compounds, notably the BHC's, DDE, DDT, chlordane and possibly Aroclor 1254 or 1260. However, at the very low levels at which they were present, it was not possible to generate spectra which could be matched with those of the reference standard materials. With extensive use of RSIC's and the calculation of relative intensities, it should be possible to confirm the presence of these compounds. This approach, however, would take a great deal of time and was not within the scope of this program.

Cleanup on Bio-Beads in which DCM is used as the mobile phase has been included as part of the proposed method in the appendix. DCM as the eluant provides a single extract which is adequate for quantitative determination by GC/ECD and is satisfactory on some sludges for confirmation by GC/MS. If the interferences in the DCM extract do not permit GC/MS confirmation, the cyclohexane procedure should then be applied to the DCM extract before GC/MS confirmation. Cyclohexane as the eluant apparently minimizes the background interferences to a somewhat greater extent than the DCM procedure and allows confirmation of organic identity by GC/MS at lower levels on difficult sludges.

A Dayton digested sludge was spiked with the Group I compounds at the 30% level and extracted in the usual manner. After cleanup with mercury, the extract was fractionated on Bio-Beads S-X2 with cyclohexane as the mobile phase and eight 25 mL fractions were caught. Each of the fractions was chromatographed on the GC/MS and the resultant chromatograms of Fractions 2 through 7 are shown as Figure 7. The complexity of these fractions is readily apparent as is the changing nature of the compound mixtures as the elution proceeds. From TIC's such as these, a retention time match can be found for any one of the compounds sought.

From the elution profiles shown in Figure 4, it was determined that the bulk of the Group II compounds should be contained in Fractions 5 through 7. Characteristic ions of the pesticides were searched and the SIC's for the ions 219, 241 and 263 in Fraction 6 are shown in Figure 8. These ions are indicative of aldrin, endrin, dieldrin, the BHC's and endosulfans. Most of the characteristic ions for these pesticides can be found in several fractions--even those in which the pesticides are not expected to be present. The single ion responses then were matched with retention times of standards and are shown on the chromatograms.

Mass spectra were extracted from the indicated points and corrected by the subtraction of background spectra. As seen in Figure 6, however, this process results in mixed spectra because of the many overlapping compounds present at any given point. Only the presence of aldrin and dieldrin was tentatively confirmed in this way. At the higher 100% spike level, the confirmation process was much less tentative. These methods are in initial stages of development and will require refinement before being used routinely for identity confirmation at very low concentration levels.

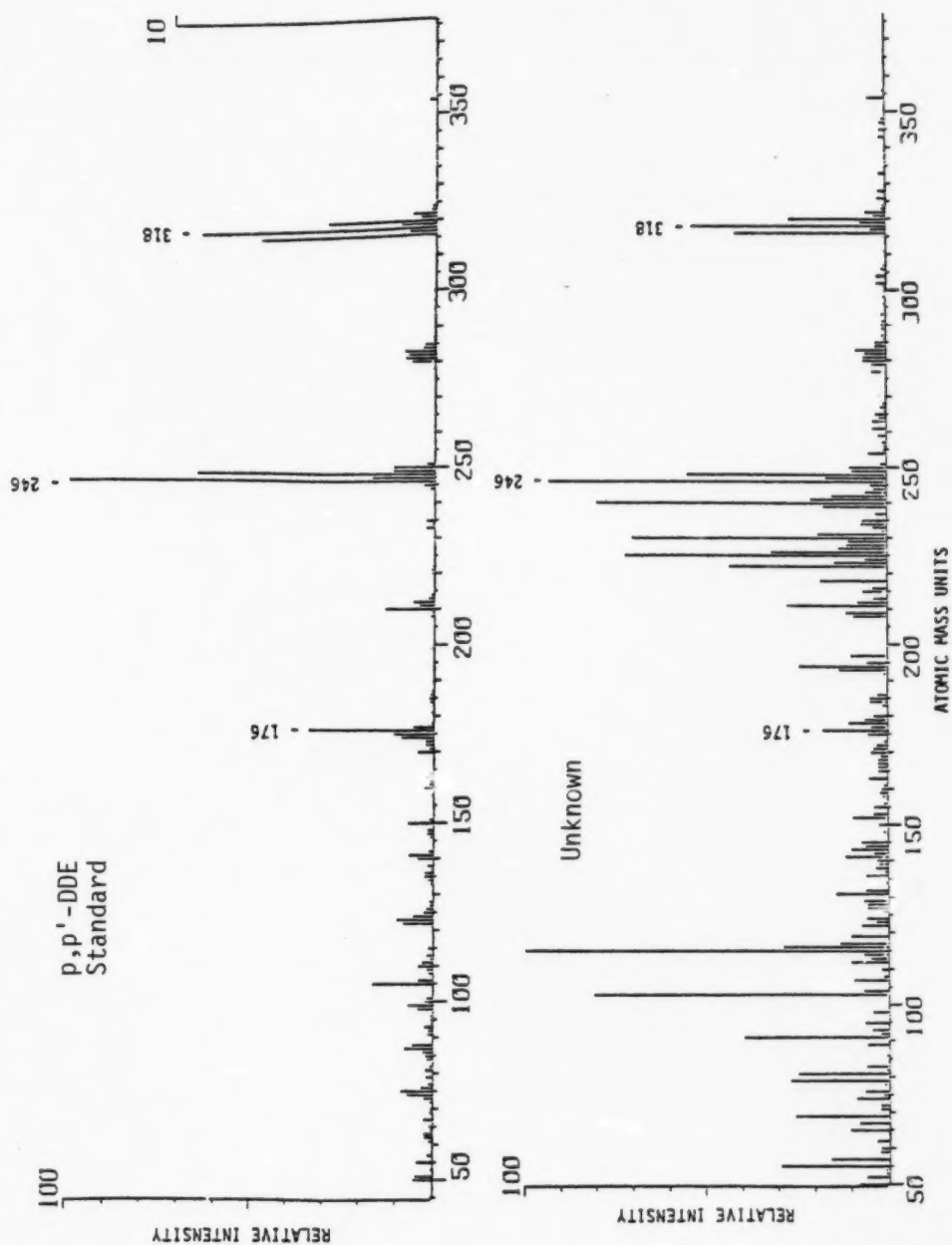


Figure 6. Mass spectrum of unknown compound extracted from elution point of p,p'-DDE

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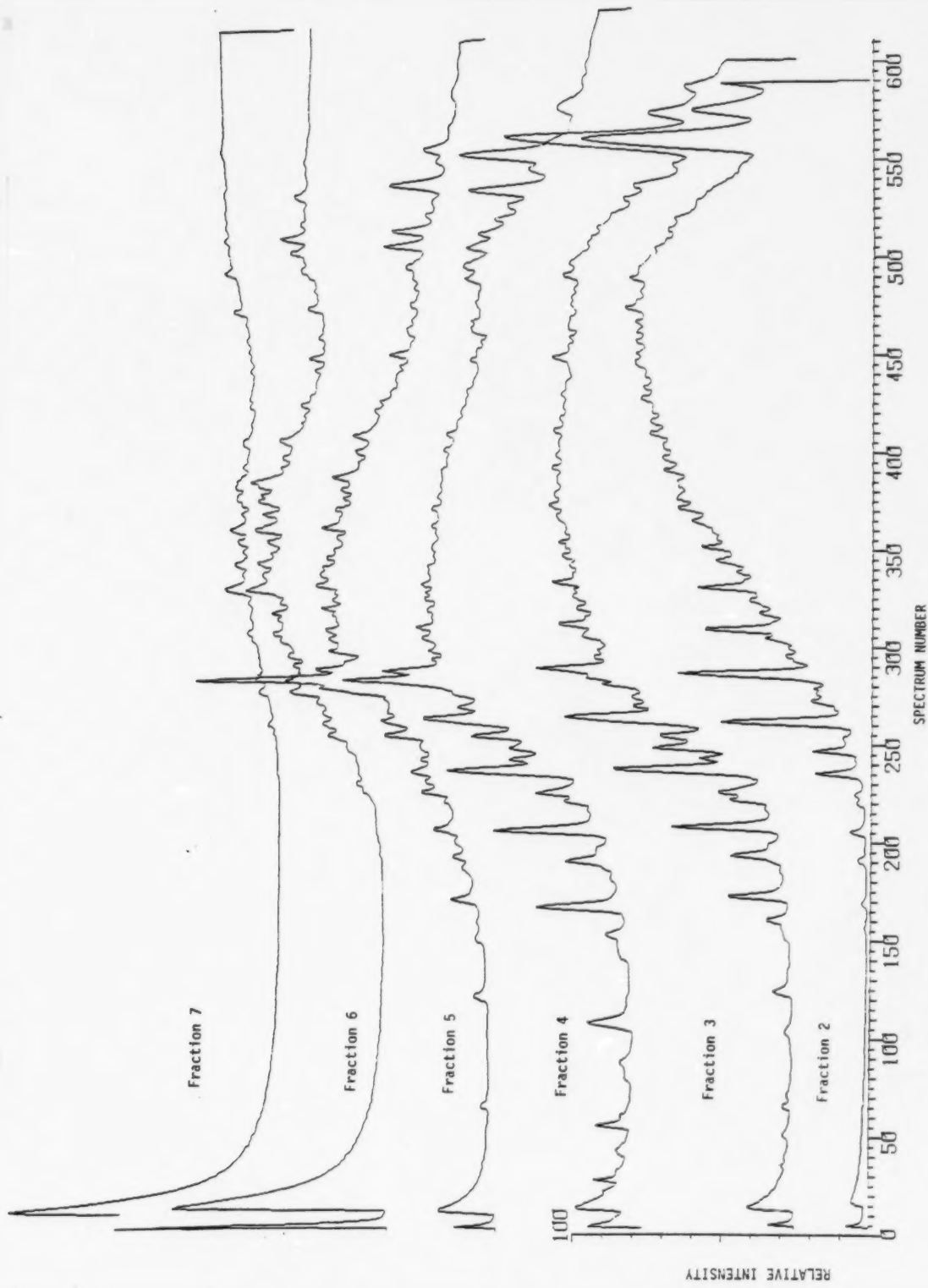


Figure 7. Total ion chromatograms from GPC Fractions

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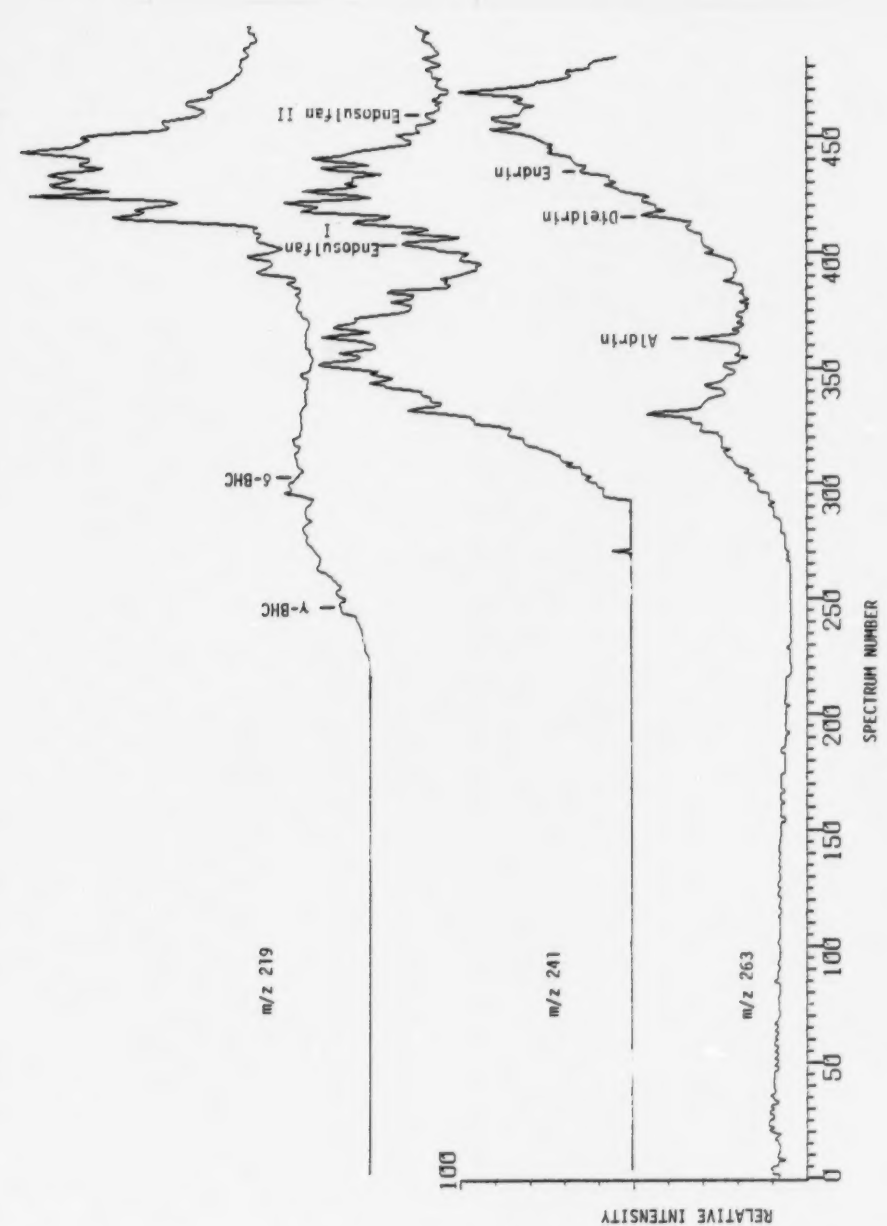


Figure 8. Single ion chromatograms from GPC Fraction 6

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THE METHOD

The procedures reported have been formulated as a recommended method for the screening of municipal sewage treatment plant sludges for compliance with the NPDES Permits Program. The method, as detailed in the appendix, includes the following elements:

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1. isolation of the organochlorine compounds in an organic matrix by extraction with a mixed solvent;
2. cleanup of the extract by:
 - a. gel permeation chromatography to remove some interfering organic compounds, and
 - b. removal of elemental sulfur by precipitation with mercury;
3. concentration by evaporation to bring the organochlorine compounds to the detectability range of the determinative procedure;
4. quantitative determination of the organochlorine compounds by gas chromatography with electron capture detection; and
5. confirmation of detected compound identities by gas chromatography/mass spectrometry with provisions for further cleanup by GPC if required.

The complex nature of sludges requires a comprehensive method that provides adequate analytical alternatives to contend with difficult situations produced by samples not conforming to expected characteristics. For this reason, it is recommended that the method be applied by experienced pesticide analysts or other similarly qualified persons.

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APPENDIX

MX00-METHOD FOR THE ANALYTICAL DETERMINATION OF CHLORINATED PESTICIDES AND POLYCHLORINATED BIPHENYL IN MUNICIPAL SLUDGE BY GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

X10 SCOPE AND APPLICATION

X11 This method covers the determination of various chlorinated hydrocarbon pesticides and a polychlorinated biphenyl by gas chromatography and electron capture detection. The complete list of compounds included in this procedure is provided in Table X11A.

X12 This method is applicable to the analytical determination of these compounds in the sludge from municipal wastewater treatment plants. It can be used to meet the monitoring requirements of the National Pollutant Discharge Elimination System (NPDES). As such, it presupposes a high expectation of finding specific compounds of interest. If the user is attempting to screen samples for all of the compounds in Table X11A, reference should first be made to Section X81 for general guidance.

X20 SUMMARY

X21 The method offers limited analytical alternatives; their use is dependent on the specific compound(s) of interest and the nature and extent of interferences. The procedure describes the use of liquid-liquid extraction with a moderately polar solvent mixture to minimize coextraction of interferences while efficiently recovering the compounds of interest. Selected general purpose cleanup procedures are included to aid in the elimination of interferences. Chromatographic conditions are suggested for the qualitative and quantitative determination of the compounds.

X22 This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons. All personnel that use this method should be thoroughly familiar with the principles of analytical pesticide determinations in the manual, "Analysis of Pesticide Residues in Human and Environmental Samples," Ed.: J. F. Thompson, June 1977.

X30 APPARATUS AND REAGENTS

X31 For sample extraction, Section X50:

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- X31.1 Neutralizing solutions-- $\text{H}_2\text{SO}_4/\text{H}_2\text{O}$ (1:2) and 10% (w/v) aq NaOH.
- X31.2 Pipette, transfer, 50 mL.
- X31.3 Kuderna-Danish (K-D) glassware
 - a. Synder column--three ball macro
 - b. Evaporation flask--500 mL and 250 mL
 - c. Receiver vial--10 mL, graduated
 - d. Vial stopper
 - e. Boiling stones--Hengar, plain, 10 mesh for micro determinations
- X31.4 Centrifuge tube--screw cap, 250 or 200 mL
- X31.5 Teflon film--1 mil, 4 inches wide by 100 foot long, cut to 4" squares
- X31.6 Extracting solvent--hexane, dichloromethane, acetone (83:15:2), all solvents pesticide grade, redistilled in glass, if necessary
- X31.7 Sodium sulfate--pesticide grade, granular, anhydrous, extracted 48 hours with dichloromethane, air dried and conditioned at 100°C for 18 hours, wash with hexane just prior to use
- X31.8 Stirrer--paddle with variable speed motor
- X31.9 Salt solution--NaCl, saturated in purified water; if required the reagent grade NaCl is cleaned up in the same manner as the Na_2SO_4
- X31.10 Steam bath with drain so condensate does not collect in the bath.

X32 For quantitation, Section X60:

- X32.1 Gas chromatograph--equipped for on-column injection
- X32.2 Glass column--1.8m long x 4mm ID packed with 1.5% SP-2250/1.95% SP-2401 on 100/120 mesh Supelcoport
- X32.3 Recorder--potentiometric strip chart (25 cm) compatible with detector
- X32.4 Syringe--10 μL volume. Additional sizes will be required
- X32.5 Reference materials--assayed quantity of compound(s) of interest, 1 $\mu\text{g}/\mu\text{L}$ stock solution in isooctane (except β -BHC in acetone) and appropriate dilute solutions
- X32.6 Computer--integrating, interfaced to the gas chromatograph, optional

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X32.7 Carrier gas--zero grade, high-purity, organic and oxygen-free, filtered through 13X molecular sieve and a catalytic oxygen scrubber.

X33 For removal of interferences, Section X70:

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X33.1 Gel Permeation Chromatography (X72)

- a. Chromatographic column--Pyrex (22 mm ID x 400 mm long) with coarse fritted disc at bottom and a stopcock equipped stem with a Teflon plug
- b. Bio-Beads--Grade S-X2 (200-400 mesh) a spherical, porous styrene-divinylbenzene copolymer with 2% crosslinking, available from BIO-RAD Laboratories, Richmond, California. Before use swell the gel by soaking in dichloromethane
- c. Dichloromethane--pesticide quality, redistilled in glass if necessary
- d. Cyclohexane--pesticide quality, redistilled in glass if necessary
- e. Inert gas--zero grade, high purity, organic free, dry; as a precaution should be filtered through 13X molecular sieve
- f. Hexane--pesticide quality, redistilled in glass, if necessary.

X33.2 Sulfur removal (X73)

- a. Mercury--triple distilled
- b. Shaker--reciprocating, variable speed
- c. Mixer--Vortex tube.

X40 SAMPLING AND PRESERVATION

X41 Samples should be collected in quantities of 1000g or more and stored in glass containers equipped with an inert liner (teflon or foil) in the cap. The container should be prewashed, solvent rinsed, air- and oven-dried before use to minimize interferences. Conventional sampling practices should be followed, except that the bottle must not be prewashed with sample before collection. Since municipal sludge is a complex mixture, usually containing oily materials, there is a significant probability that these oils would adhere to the sample container. These oils would be expected to selectively dissolve chlorinated pesticides thereby changing concentrations in the sample.

X42 Generally, samples should be analyzed as soon as possible after sampling. Since this may not be ordinarily possible, the sample

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should be refrigerated or iced immediately after collection and maintained at 5°C until extraction which should not be delayed any longer than absolutely necessary. An acceptable alternative to storage of samples is immediate extraction and cold storage of the extract in the dark. Decomposition in the extracts can be evaluated by storing spiked controls along with the samples.

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X50 SAMPLE EXTRACTION

- X51 Stir the sample and allow to come to room temperature. Neutralize to $\text{pH } 7.0 \pm 0.5$ with dilute H_2SO_4 or NaOH solution. While the sample is being stirred to keep the solids distributed, withdraw and weigh a 20 g analytical sample. Transfer the analytical sample to a 200 mL centrifuge bottle containing 20 mL saturated NaCl solution.
- X52 Add 60 mL extracting solvent, cover the mouth of the centrifuge bottle with Teflon film and affix the screw cap; shake for two minutes or agitate one minute with a high speed, immersible probe homogenizer.
- X53 Place the bottle(s) in a refrigerated centrifuge maintained at 15°C and spin at 500 x G for 20 minutes.
- X54 Remove the extracting solvent, the top layer, from the centrifuge bottle with a 50 mL pipette and pass it through a chromatographic column containing 10-15 mm of anhydrous Na_2SO_4 and collect it in a 500 mL K-D flask equipped with a 10 mL receiver vial.
- X55 Add 60 mL extracting solvent to the centrifuge bottle and repeat the procedure adding the extract to that in the K-D apparatus.
- X56 Perform the extraction procedure a third time in the same manner.
- X57 Add a clean boiling chip to the extract; attach a three-ball Synder column to the evaporator and support the apparatus in a steam bath. Concentrate the extract to 1-2 mL in the receiver for 10-20 minutes and remove from the steam bath. The condensed vapor will drain into a final volume of 5-10 mL in the receiver.
- X58 Remove the Synder column and rinse the extractor flask into the receiver with 1-2 mL hexane. A 5 mL syringe is recommended for this operation.
- X59 Stopper the receiver and store at 5°C in a dark location while awaiting chromatography.

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- X61 Quantitative measurements called for by this method are made by gas chromatography and electron capture detection. The electron capture detector provides some selectivity to certain molecules such as alkyl halides, conjugated carbonyls, nitriles, nitrates and polycyclic aromatics and response is so low as to be virtually nonexistent to hydrocarbons, alcohols, unconjugated carbonyls and similar molecules. Response is the greatest to halogen and nitroso compounds and increases sharply for multiple halogen substitution, thereby enhancing selectivity to a great degree for the compounds listed in Table X11A. Use of the electron capture detector requires cleanup procedures prior to quantitation to remove organic interferences and elemental sulfur. The column packing recommended for use with this method provides resolution adequate for the quantitative determination of the compounds listed in Table X11A.
- X62 The electron capture detector must be operated within its linear response range and at a noise level less than 1% full scale. A final dilution of 10 mL extract from a 20 g wet sample is optimum as any greater concentration of extract without further cleanup overwhelms the detection system and can render it inoperative for as long as a day.
- X63 Calibration and spiking solutions are made from the 1 $\mu\text{g}/\mu\text{L}$ stock solutions of the individual standards. Dilutions are made with hexane such that an injection of 10 μL will contain the appropriate Group I or Group II standard(s) in the amounts of 0.3 and 1.0 ng; 3 and 10 ng Group III; 15 and 50 ng Group IV and 6 and 20 ng Group V. Stock solutions are diluted with acetone such that 1 mL will contain the appropriate amount of standard(s) for spiking the wet sludge with the desired amount. If the analytical determination is being made of more than one compound in Group I or Group II the procedure may be simplified by mixing the compounds to make one spiking solution. The amount of compound spiked in 20 g wet sludge is 0.3 and 1 μg of each of the compounds in Group I and II; 3 and 10 μg total of Group III; 15 and 50 μg total of Group IV, and 6 and 20 μg total of Group V.
- X64 Organohalide pesticides--The retention times relative to that of aldrin of the 17 pesticides are listed in Table X64A. The four peaks listed for Group II and three peaks listed for Group IV are the most representative ones noted in the chromatograms of those formulations. It is apparent from these retention times that certain combinations of pesticides will result in overlapping chromatographic peaks; hence, unambiguous identifications cannot always be made. The procedures given in this method for the removal of interferences will only partially resolve these anomalies. In order to obtain data from the quantification of coeluting compounds, it is necessary that an experienced pesticide analyst apply these procedures and others, such as

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column adsorption chromatography and gas chromatography/mass spectrometry both scanning and single ion monitoring.

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X65 Chlorinated hydrocarbons--The relative retention times of five peaks of the polychlorinated biphenyl listed in Table X65A are representative of this multicomponent formulation. The analyst may key on these peaks to determine the presence of AR 1260 in sludge extracts in combination with the comparison to the entire chromatogram of an authentic standard. As in X64, the presence of other chlorinated hydrocarbons which coelute with known compounds in the AR 1260 will increase the difficulty of identification and quantification. In this instance, it will be necessary to use additional GC/MS techniques from this method and others from the scientific literature to enhance the probability of unequivocal identification.

X66 General comments--The determination of any of the chlorinated hydrocarbons within the scope of this method requires the application of an appropriate cleanup procedure such as the gel permeation chromatography technique. This cleanup removes a significant amount of organic extractable substances thereby eliminating them as interferences which would make it virtually impossible to use electron capture detection for the analysis of municipal sludge. The extracts prepared in this manner are suitable for confirmation of identity by GC/MS or, if a cleaner extract is required, the more retentive GPC cleanup (X74) may also be used. Method MX00 has been qualified for one polychlorinated biphenyl, AR 1260, but it is of equal applicability to those listed in Table X66A. The response characteristics for each formulation would have to be determined by gas chromatography/electron capture detection prior to analytical determination in sludge.

X70 REMOVAL OF INTERFERENCES

X71 The procedures in this section have proven utility in the analysis of municipal sludges for the determination of polychlorinated hydrocarbons. Gel permeation chromatographic fractionation allows the electron capture detector to be used for chromatographic detection as does the removal of elemental sulfur with mercury. GPC also provides cleanup which facilitates confirmation of compound identity by GC/MS; for further fractionation and enhancement of GC/MS the more adsorptive GPC procedure in X74 may be used after cleanup.

X72 GPC provides cleanup by eliminating high molecular weight materials which are not retained on the gel as are the compounds of interest. The response of interferences is decreased so the polychlorinated hydrocarbons may be recognized by electron capture detection.

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- X72.1 Cover 30-40 g Bio-Beads S-X2 with DCM and let stand overnight to swell the polymer.
- X72.2 Place a glass wool plug over the fritted disc of the chromatographic column; rinse the column and glass wool with DCM and tap down with a glass rod.
- X72.3 Close the stopcock and add enough DCM to cover the glass wool.
- X72.4 Add the swollen gel in small portions to the column while draining excess DCM and tapping the sides of column; continue until a well-packed gel column 30-31 cm in height results.
- X72.5 Carefully drain the DCM to just expose the top of the gel; add a 15-20 mm layer of anhydrous, granular Na_2SO_4 and sufficient DCM to cover it.
- X72.6 Adjust the sample extract volume to less than 3 mL in DCM.
- X72.7 Drain the DCM from the column to just expose the top of the gel; quantitatively transfer the extract to the gel bed keeping the volume as small as possible; drain the column to just expose the gel bed below the Na_2SO_4 and close the stopcock.
- X72.8 Add DCM to the column to a level of 20-40 mm above the Na_2SO_4 ; maintain this level throughout the elution.
- X72.9 Open the stopcock to begin solvent flow at a rate of 3 mL/minute under these conditions.
- X72.10 Discard the first 90 mL eluted.
- X72.11 Collect the next 25 mL of eluate in a 250 mL K-D flask equipped with a 10 mL ampule.
- X72.12 Concentrate the 25 mL eluate of interest to 5-8 mL in the K-D evaporative concentrator, cool, add 25 mL of hexane and concentrate again to 5-8 mL.
- X72.13 Quantitatively transfer the concentrate by decantation and three 1 mL washings to a 10 mL volumetric flask and evaporate to less than 10 mL with a stream of pure, dry inert gas. Make the solution to 10 mL with hexane.

X73 Sulfur removal--Most extracts of municipal sludge are expected to contain elemental sulfur which interferes with the gas chromatographic analysis. The sulfur can be removed by shaking a portion of the extract with triple distilled mercury.

- X73.1 Quantitatively transfer a 2 mL aliquot of the extract to a 5 mL foil- or Teflon-lined screw cap vial.
- X73.2 Add 0.4 mL triple distilled mercury to the extract.

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- X73.3 Shake for approximately 10 seconds on a vortex mixer and then for 2 hours on a reciprocating shaker at 3-5 cycles per second.
- X73.4 At the end of one hour of shaking, check the condition of the mercury by taking the vial in the hand and swirling in a circular motion. If a bright mercury color is not visible at the bottom of the vial, add 0.2 mL mercury and continue shaking; otherwise, continue shaking to the end of the 2-hour period.
- X73.5 Allow the extract to settle undisturbed for a minimum of 15 minutes.
- X73.6 Withdraw 10 μ L of the organic extract with a microliter syringe without disturbing the precipitate.
- X73.7 Inject the extract into a GC/ECD for quantitative determination.

X74 Gel permeation chromatography--On Bio-Beads S-X2 with cyclohexane mobile phase will fractionate the extract and minimize some of the interferences to allow enhancement of gas chromatographic/mass spectrometric response for confirmation of identity of the polychlorinated compounds.

- X74.1 Place 25 g Bio-Beads S-X2 in a beaker and add 2-3 volumes of cyclohexane. Cover the beaker and let stand overnight.
- X74.2 Transfer the swollen gel to a glass, 200 mm diameter column equipped with a stopcock. The bed volume is approximately 50 cc.
- X74.3 Flush the column with 75-100 mL cyclohexane under an inert gas pressure of 62 kPa without letting the gel go dry.
- X74.4 Allow the cyclohexane to drain to the top of the gel bed and transfer the remaining 8 mL extract without the sulfur removed to the top of the gel bed.
- X74.5 Wash the vial and sides of the column 3-4 times with small volumes of cyclohexane allowing the solvent to drain to the top of the gel bed each time.
- X74.6 Fill the column with cyclohexane and elute at 5 mL/minute under an inert gas pressure of 62 kPa.
- X74.7 Collect eight 25 mL eluate fractions, each in a K-D evaporative concentrator.
- X74.8 Evaporate each fraction in a steam bath as in Section X57 to a volume <5 mL, being careful not to let the sample dry out.
- X74.9 Transfer each concentrate to a 0.5 mL foil- or Teflon-lined screw cap vial.

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X74.10 Concentrate each fraction to 0.1 mL under a stream of pure, dry inert gas and store refrigerated for GC/MS determinations.

X80 GC/MS CONFIRMATION OF IDENTITY

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X81 Extracts of sludge from municipal treatment plants will normally contain significant amounts of substances that respond to the gas chromatographic electron capture detector. A positive response at a retention time coinciding with that of the compound formulation listed in Table X11A cannot be considered absolute proof that such a substance is present. Confirmatory identification by gas chromatography/mass spectrometry should be used routinely for the analysis of municipal sludges.

X82 If this method is to be used to survey samples for a large number of compounds, then the verification of identity by GC/MS becomes essential.

X83 Tentative identification of the pesticides and PCB's is made by comparison of the mass spectra of the unknowns with those of authentic standards extracted from spiked sludge and recorded under similar gas chromatographic conditions.

X84 A useful guide for the GC/MS confirmation of identity may be found in the EPA manuals, "Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants," April 1977, and "An EPA Manual for Organics Analysis Using Gas Chromatography/Mass Spectrometry," March 1979, EPA-600/8-79-006.

X90 QUALITY ASSURANCE AND QUANTIFICATION

X91 The determination of quantities of the analyte substances is done by the "method of additions" using the results of analysis of the unspiked and two spiked sludges as the three data points to construct the calibration curve as shown in Figure X91A. Relatively "clean" sludge will allow the use of peak areas for the measure of response but in most cases peak heights are more reliable and it is recommended they be used unless the reliability of peak area measurements can be demonstrated.

X92 The multicomponent formulations present a problem in that each peak within a chromatogram will be different from the others in response characteristics and it is also quite difficult to get a match between standards and environmental samples. The recommended method of quantification in this instance is to use the sum of the response of peaks, which from mass spectrometric and retention data are recognized as belonging to the formulation, to construct the calibration curve.

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X93 Standard quality assurance practices should be used with this method to the extent necessary to ensure reliable results. Field replicates should be collected to validate the precision of the sampling technique and laboratory blanks and replicates should be analyzed to validate the precision of the analysis. The analysis of fortified samples can be used to validate the apparent accuracy of the analysis.

X94 Sludge from municipal sewage treatment plants is variable in organic content. Because of this, a routine should be followed throughout the program of interspersing samples fortified in the field throughout the real samples for blind analyses in the laboratory.

X100 CALCULATIONS AND REPORTING

X101 The concentration of analyte in the sample is determined as micrograms chlorinated hydrocarbon per 20 g wet sample. Determine concentration in the sample on a dry basis according to the following formula

$$\text{Micrograms/g-Solids} = \frac{C}{P \times W}$$

C = amount of analyte, in micrograms, in 20 g of sludge.

P = $\frac{\% \text{ solids in sludge}}{100}$

W = weight of sample analyzed in grams.

X102 Report results in micrograms per gram solids without correction for recovery data. All data obtained should be reported.

TABLE X11A. LIST OF SUBSTANCES DETERMINED
BY THIS METHOD

Group I	Group II
α -BHC	γ -BHC
β -BHC	Heptachlor
δ -BHC	Aldrin
Heptachlor epoxide	Endosulfan I
p,p'-DDE	Endosulfan II
p,p'-DDD	Dieldrin
p,p'-DDT	Endrin
	Endrin aldehyde
Group III	Chlordane
Group IV	Toxaphene
Group V	PCB, Aroclor 1260

TABLE X64A. RELATIVE RETENTION RATIOS OF ORGANOHALIDE PESTICIDES

Pesticide	Retention Ratio
α -BHC	0.56
γ -BHC	0.69
β -BHC	0.78
Heptachlor	0.84
δ -BHC	0.90
Aldrin	1.00
Heptachlor epoxide	1.46
Endosulfan I	1.82
p,p'-DDE	2.12
Dieldrin	2.21
Endrin	2.66
p,p'-DDD	3.20
Endosulfan II	3.23
p,p'-DDT	3.23
Endrin aldehyde	4.17
Chlordane*	1.13, 1.62, 1.75 1.75, 3.12
Toxaphene*	2.20, 3.28, 3.97

* These formulations are complex mixtures of many compounds and analytical data will vary among samples. It is essential that the analyst treat these formulations carefully and compare mass spectrometric and retention data often with that of authentic standards.

TABLE X65A. RELATIVE RETENTION RATIOS OF PCB AR 1260

Peak No.	Retention Ratio
1	2.38
2	2.63
3	2.98
4	3.36
5	3.79
6	5.95

TABLE X66A. LIST OF POLYCHLORINATED BIPHENYLS
TO WHICH THIS METHOD IS APPLICABLE*

Aroclor 1016
Aroclor 1221
Aroclor 1232
Aroclor 1242
Aroclor 1248
Aroclor 1254
Aroclor 1260
Aroclor 1262

* The polychlorinated biphenyls are multicomponent mixtures which exhibit numerous peaks over a broad retention range. It is essential to the recognition of these mixtures that the analyst thoroughly familiarize himself with the gas chromatographic and mass spectrometric characteristics of authentic standard mixtures of these formulations.

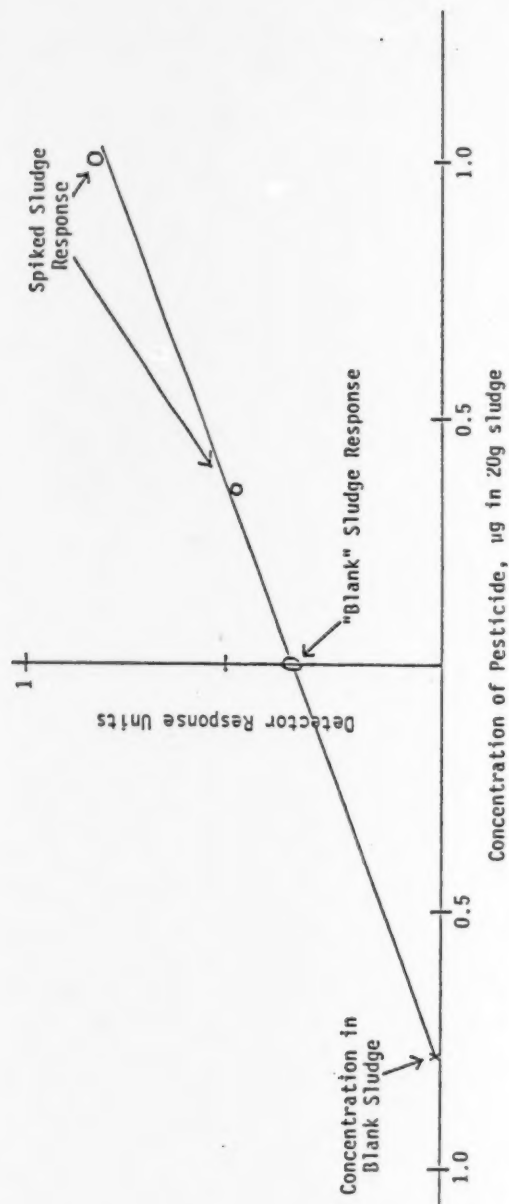


Figure X91A. Method of additions curve for the determinations of pesticides and PCB's in municipal sludge